STUDIES ON INDUCED MUTAGENESIS IN CHILLI (*Capsicum annuum* L.)

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BY

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Dedicated

To My

Beloved Family

and

Sir Syed Ahmed Khan

(Oct.17,1817-Mar.27,1898)

(Founder, Aligarh Muslim University Aligarh-INDIA)

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(Mohd Gulfishan)

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Chapter-1

INTRODUCTION

INTRODUCTION

Mutation, a term coined by De Vries (1901) for the appearance of new phenotype in *Oenothera lamarkiana*, is the sudden heritable change in the genotype of an organism. Mutation is the ultimate source of all genetic changes which provide the raw material for evolution and is a very valuable approach for improvement of economic characters in plants. It is a process by which the genetic information is changed in stable manner either in nature or experimentally by the use of chemicals or radiations. These chemicals and radiations, with the quality of acting upon the genetic material are called mutagens. Radiations, also called physical mutagens, include Ionizing Radiations e.g., α-rays, β-rays, fast neutrons, thermal neutrons, x-rays, and γ-rays, and Non Ionising Radiation: e.g., ultraviolet (UV) radiation. Chemical mutagens includes alkylating agents such as Ethyl methane sulphonate (EMS) Methyl methane sulphonate (MMS) Ethylene imines (EI), Diethyl Sulphate (DES) etc., Azides e.g., sodium azide, acridine dyes e.g., Acriflavine, Acridine orange, proflavin etc., base analogues e.g., 5 Bromouracil, 2 Aminopurine, 5 chlorouracil, etc., and other direct acting chemicals such as nitrous acid, mustard gas etc.

Physical mutagens predominantly induce chromosomal aberrations either through ionization of the target itself or indirectly through mutagenic free radicals as a result of ionization of background components. Chemical mutagens have shown evidence of more specific action than the physical mutagens, which act at random. Many cases of mutagenic specificity have been found to be regional (with reference to the chromosome) rather than genic. Though, there were several attempts to induce mutations by chemical agents (Westergard, 1957; Gustafsson, 1969), the first definite evidence that chemical agents can induce mutations was given by Auerbach and Robson (1942) in *Drosophila* by using mustard gas and Oehlkers (1943) in *Oenothera* by urethane.

It was clear from the pioneering studies in Sweden that alkylating agents are particularly suited for mutagenic studies in plants (Ehrenberg & Gustafsson, 1957; Ehrenberg, 1960). US, Europe, Japan and China emerged as great centers for induced mutagenesis work in 1950s on this planet (Chopra, 2005) and at the same time Swaminathan and his co-workers also initiated studies on chemical mutagenesis in crop plants at the Botany Division, Indian Agriculture Research Institute (IARI), India (Natarajan, 2005).

It is well established that mutagenic agents are effective for inducing genetical changes in the treated population of different plant species including *Triticale* (Viswanathan & Reddy, 1998), *Lens culineris* (Reddy & Annadurai, 1992; Verma *et al* 1999), *Vigna radiata* (Khan *et al*, 1999), *Triticum aestivum* (Kalia *et al*., 2000), *Vicia faba* (Khan *et al*., 2005a, b; 2006a, b), *Trigonella foenum-graecum* (Jabee *et al*., 2007) and *Helianthus annuus* (Khurseed *et al*., 2009), *Capsicum annuum (*Abdul Salam *et al*., 2010; *Sri Devi et al., 2011).*

The use of induced mutation over the past six decades has played a major role in the development of superior plant varieties all over the world. After the epoch making discoveries made by Mullar (1927) and Stadler (1928), a large amount of genetic variability has been induced by various mutagens in most economically important plant species, and a small portion of those induced variations has resulted in the development of more than 3000 mutant varieties worldwide in about 180 plant species during the past 60 years (Shu and Lagoda 2007). Among the mutant varieties the majority are of food crops.

Induced mutations are considered as an alternative to naturally occurring genetic variation that serves as the source of germplasm for crop improvement programme and also an alternative to hybridization and recombination in plant breeding since mutations gives rise to non-existing variations (Brock, 1970). It is an effective tool in hands of plant breeders specially in crop with narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding programme.

The inheritance of important economic trait such as yield, quality, adaptation, pest and stress resistance, upon which rests of much of the future of plant improvement can be understood through the analysis of a wide range of induced mutations. These manifestation are usually due to: a) chromosomal rearrangement or b) gene mutation or c) both (Gustaffson,1965; Gaul 1965). Induced mutation is the best method to evolve new cultivars by producing genetic variability at gene level. The application of mutagenesis in agriculture for improving the crop replaces the conventional breeding methods where natural variability present either in the base population initially or introduced through hybridization, is subjected to recombination and selection so as to increase the frequency of favourable combination of genes in the selected line. Mutation breeding helps in creation of new gene alleles that do not exist in the germplasm; induction of new gene alleles for a commercial variety so new varieties carrying desired mutation alleles can be directly used as commercial variety. The limited genetic changes of any single plant of a mutated population and the often recessive nature enable breeders to develop a new variety in a short period of time.

The success in the plant improvement programme, however, depends basically on controlling and directing the induced mutation process for the production of desired mutations. Only through a careful screening and selection programme the magnitude of genetic variability induced by different mutagens could be exploited for obtaining the desirable lines.

Mutations provide an opportunity to create hitherto unknown alleles so that the plant breeding does not remain handicapped because of limited allelic variation at one or more gene loci of interest. Mutation breeding has helped to rectify certain specific defects in otherwise acceptable cultivars. Induced mutations increase genetic variability for certain characters so that selection is more effective and the probability of getting the desired variability is obviously urgently needed, so that suitable type of crop plants may be developed. Fried (1969) concluded that for increasing food production in the world, induced

mutagenesis is important in creating variability in the breeding population to improve yield, earliness, disease resistance, lodging resistance etc. Induced mutations will continue to play an increasing role in creating crop varieties with traits such as modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of environmentally sustainable agriculture. Future research on induced mutations will also be important in the functional genomics of many food crops.

1.1 The Indian Contribution to Mutation Breeding:

Major research centers made important contribution in the field of induced mutation and development and release of mutant varieties are Indian Agricultural Research Institute (IARI) in New Delhi, Bhabha Atomic Research Center (BARC) in Mumbai, ICRISAT, Hyderabad, Tamil Nadu Agricultural University (TNAU) in Coimbatore, and National Botanical Research Institute (NBRI) in Lucknow. According to Kharkwal and Shu, (2009), 343 mutant cultivars belonging to 57 plant species were approved and released in India. Detailed information regarding number of mutant varieties released for cultivation in India and total number of varieties released in each crop is presented Table 1.

Table 1: Mutant Varieties Released in India.

S.N	Latin name	Common name	No. of var.	S. N.	Latin name	Common name	No. of var.
1.	*Ablemoschus esculentus* L. Moench	Okra	2	30	*Matricario commomilla*	German chamomile	1
2.	*Arachis hypogaea* L.	Groundnut	18	31	*Mentha spicata*	Spearmint	1
3.	*Bougainvillea spectabilis* Wild	Bougainvillea	13	32	*Momordica charantia* L.	Bitter gourd	1

4.	*Brassica juncea* L.	Mustard	9	33	*Morus alba* L	Mulberry	1
5.	*Cajanus cajan* L. Millsp	Pigeonpea	5	34	*Nicotiana tabacum* L	Tobacco	1
6.	*Capsicum annuum* L.	Green pepper	1	35	*Orazya sativa* L	Rice	42
7.	*Carica papaya* L. 1	Papaya	1	36.	*Papaver somniferum* L.	Opium poppy	2
8.	*Chrysanthemum* sp.	Chrysanthemum	49	37	*Pennisetum typhoides* L.	Pearl millet	5
9.	*Cicer arietinum* L.	Chickpea 8	8	38	*Phaseolus vulgaris* L.	Freneh bean	1
10.	*Corchorus capsularis* L.	White Jute	2	39	*Pisum sativum* L	Pea	1
11.	*Corchorus olitoirus* L.	Tossa Jute	3	40	*Plantago ovata* L.	. Isabgol	2
12.	*Curcuma domestica* Val.	Turmeric	2	41	*Polyanthus tuberosa* L.	Tuberose	2
13.	*Cymbopogon winterianus* Jowitt.	Citronella	9	42	*Portulaca grandiflora* L.	. Portulaca	11
14.	*Cyamopsis tetragonoloba* L.	Cluster Bean	1	43	*Ricinus communis* L.	Castor	4
15.	*Dahlia* sp.	Dahilia	11	44	*Rosa* sp.	Rose	16
16.	*Dolichos lablab* L.	Hyacinth bean	2	45	*Sachharum officinarum* L.	Sugarcane	9
17.	*Eleusine coracana* L.	Finger millet	7	46	*Sesamum indicum* L.	Sesame	5
18.	*Gladiolus* L.	Gladiolus	2	47	*Setaria italica*	Foxtail millet	1

					L.		
19.	*Glycine max* L/ Merr.	Soybean	7	48	*Solanum khasianum* Clarke	Khasianum	1
20.	*Gossypium arboreum* L.	Desi cotton	1	49	*Solanum melongena* l.	Brinjal	1
21.	*Gossypium hirsutum* L.	American cotton	8	50	*Solenostemon rotundifolius*	Coleus	1
22.	*Helianthus annus* L.	Sunflower	1	51	*Trichosantheus angui*	Snake gourd	1
23.	*Hibiscus sinensis* L.	Hibiscus	2	52	*Trifolium alexandrium* L.	Egyptian clover	1
24.	*Hordeum vulgare* L.	Barley	13	53	*Triticum aestivum* L.	. Wheat	4
25.	*Hyocyamus niger*	Indian henbane	1	54	*Vigna aconitifolia* Jacq. M.	Moth bean	5
26.	*Lantana depressa* L.	Wild sage	3	55	*Vigna mungo* L. Hepper	.Blackgram	9
27.	*Lens culinaris* L. Medik	Lentil	3	56	*Vigna radiata* L. Wiczeck	Mungbean	15
28.	*Luffa acutangula* Roxb.	Ridged gourd	1	57	*Vigna unguiculata* L.walp	Cowpea	10
29.	*Lycopersicon esculentum* L.	Tomato	4	**Total Number of varieties released**			**342**

Source: Kharkwal and Shu, (2009).

Mutation breeding has made significant contribution in increasing the production of rice, groundnut, castor, chickpea, pigeonpea, mungbean and urdbean in Indian subcontinent. *Kharkwal et al.*, (2001) made survey of mutation breeding and related literature and suggested that the desirable results were obtained from the induced variability when it was fully integrated with conventional crop breeding programs. Thus Indian plant breeding made valuable contribution in achieving the target of self-sufficiency in food production and strong economic growth of the country.

Table 2: Mutant varieties of different crops released for cultivation in India.

Crop	No. of varieties	Specific crop and no. of varieties
Cereals	**74**	rice (42), barely (13), pearl millet (5), finger millet (7), foxtail millet (1), wheat (4), sorghum (2)
Pulses	**57**	mungbean (15), blackgram (9), chickpea (8), cowpea (10), moth bean (5), pea (1), pigeonpea (5), French bean (1) lentil (3)
Oilseeds	**44**	groundnut (18), mustard (9), castor bean (4), sesame (5), soybean (7) sunflower (1)
Fibre crops	**14**	American cotton (8), tossa jute (3), white jute (2), desi cotton (1)
Vegetables	**14**	tomato (4), turmeric (2), bitter gourd (1), brinjal (1), green pepper (1), okra (2), ridge gourd (1), snake gourd (1), cluster bean (1)
Cash crops	**10**	sugarcane (9), tobacco (1)
Medicinal crops	**17**	citronella (9), German chamomile (1), Indian henbane (1), isabgol (2), khasianum (1), opium poppy (2), Spearmint (1)
Fruit trees	**2**	mulberry (1), papaya (1)
Forage crops	**1**	Egyptian clover (1)
Ornamentals	**110**	Chrysanthemum (49), rose (16), dahlia (11), portulaca (11), bougainvillea (13), wild sage (3), gladiolus (2) Hibiscus sp. (2), tuberose (2) Coleus (1)
Total	343	

Source: Kharkwal and Shu, (2009).

The annual income increased about 50 million US $ due to development and cultivation of mutant varieties (Amano, 1997). More than Rs. 258 crores of income was generated by India due to release of 343 mutant cultivars belonging to 57 plant species (Kharkwal and Shu, 2009).

1.2 DESCRIPTION OF *CAPSICUM ANNUUM* L.

1.2.1 Origin:

The genus *Capsicum* L. is in the large family Solanaceae. It consists of about 25 wild and 5 domesticated species (Bosland and Votava, 2000). The five variously domesticated species are *Capsicum annuum* ,*C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens* (Heiser, 1985). The centre of diversity for *Capsicum* is in south-central South America (Eshbaugh, 1980,), with the majority of species having some range in Brazil and/or Bolivia. Some of the non-domesticated species are gathered for occasional use. The primary centre of origin for domesticated *C. annuum* is in semitropical Mexico (Andrews, 1995). The four other domesticated species are usually believed to have originated in South America (Eshbaugh *et al*.,1983). There is an archaeological evidence from about 9000 BC for the use and subsequent domestication of *Capsicum annuum* in central-eastern and south-central Mexico in the states of Tamaulipas (near Ocampo), Puebla (Tehuacán Valley) and Oaxaca (Guilá Naquitz) (Bosland, 1996). The ready appeal of *Capsicum* was such that within half a century it had been distributed as far as Asia, and it has been integrated and continues to be diversified in cultures worldwide as it had been originally in the Americas (Eshbaugh, 1983; Yamamoto and Nawata, 2005).

1.2.2 Taxonomy, Morphology and Floral Biology:

Capsicum annuum L. is a dicotyledonous flowering plant with many general names in English, such as hot pepper, chili, chilli or chile pepper, and as well sweet pepper and bell pepper. Sometimes the plant is just called pepper, which however is often reserved for the earlier known Asian *Piper nigrum* (black pepper, white pepper) in the family Piperaceae. The systematic position

of *Capsicum annuum* L. according to Bentham and Hooker's(1862-1883) system of classification is as follows:

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* –Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Gamopitalae*
Order	*Polimoniales*
Series	*Bicarpillatae*
Family	*Solanaceae* – Potato family
Genus	*Capsicum* L. – pepper
Species	*Capsicum annuum* L. cayenne pepper

Capsicum annuum L. is usually grown as a herbaceous annual in temperate areas. However, ecologically it is a perennial shrub in tropical areas, and it can be grown as a perennial in climate-controlled greenhouses. This species includes the vast majority of the cultivated pungent and non-pungent (sweet) *Capsicum* peppers in temperate as well as some tropical areas. Stem is erect branched from base. Leaves are simple, alternate, ovate, exstipulate, mucronate. Flower are opposite, bisexual, actinomorphic, hypogynous and usually pentamerous with a diameter of diameter of 9-15 mm (Bosland and Votava, 2000). Flowers are complete, with calyx, corolla, and male and female sex organs. Calyx is broadly campanulate, ribbed, about 2 mm long, and truncate or undulate to weakly or prominently dentate with 5-7 teeth. Corolla is fused, short-tubed with usually 5 petals. Fruit is a berry. There is extensive diversity in fruit shape, size, wall thickness and fleshiness, colour and pungency, determined by genetic and environmental factors. The seed develops

from a campylotropous ovule (Dharamadhaj and Prakash, 1978). An average *C. annuum* seed is about 5.3 mm long, 4.3 mm wide and 1 mm thick, with a surface area of 33 mm2 (Chen and Lott, 1992). *C. annuum* is a partially self-pollinating crop (Allard, 1960); wind or similar mechanical disturbance may enhance self-pollination (Raw, 2000; Kristjansson and Rasmussen, 1991)..

1.2.3 Cytology:

Capsicum species are diploid, mostly with most having 24 chromosomes ($n = x = 12$), but with several wild species having 26 chromosomes ($n = x = 13$) (Pickersgill, 1991; Tong and Bosland, 2003). *Capsicum annuum* has 24 chromosomes; usually 2 pairs (or sometimes 1) are acrocentric, and 10 (or 11) pairs metacentric or sub-metacentric (Lanteri and Pickersgill, 1993). Its nuclear DNA content (determined by flow cytometry and Feulgen densitometry) has been reported to have a mean 1C-value of 3.38 picograms per nucleus, which Moscone *et al.* (2003) discussed in relation to other reports with varying methodology that ranged from 2.76 to 5.07 pg per nucleus. The total length of the chili pepper genome has been estimated to be between 1498 cM and 2268 cM, which is approximately two to three times larger than tomato genome (Kang *et al.*, 2001;).

1.2.4 Nutritive Value and Medicinal Importance:

In the species *Capsicum annuum* L., throughout the world, there is phenotypic diversity in plant habit and especially in shapes, sizes, colours, pungency, and other qualities of the fruits (Andrews, 1995). This immense horticultural, agricultural and biological diversity has helped to make *C. annuum* globally important as a fresh and cooked vegetable (*e.g.* for salads, warm dishes, pickled etc.) and a source of food ingredients for sauces and powders and as a colourant, which is used as well in cosmetics (Andrews, 1995, Bosland and Votava, 2000). Chili pepper comprises numerous chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre, and mineral elements (Bosland and Votava, 2000). *Capsicum* is rich in vitamin C (ascorbic acid) and Zinc, two nutrients which are vital for a

strong and healthy immune system. It is also high in vitamins, A, C, rutin (a bioflavonoid), beta carotene, iron, calcium and potassium. *Capsicum* also contains magnesium, phosphorus, sulphur, B-complex vitamins, sodium and selenium, Fats: 9-17%, Proteins: 12-15%.The capsaicinoids are alkaloids that give hot chili peppers their characteristic pungency. The rich supply of carotenoids contributes to chili pepper's nutritional value and colour (Britton and Hornero-Méndez, 1997).

Moreover, the species is used medicinally and medically, and provides the ingredient for a non-lethal deterrent or repellent to some human and animal behaviours (Krishna De, 2003). *Capsicum* stimulates everything from blood flow to peristaltic action in the stomach, to intestinal transit time. *Capsicum* exerts a variety of desirable actions on the entire cardiovascular system. It has the extraordinary ability to enhance cardiovascular performance while actually lowering blood pressure. (Lee *et al*, 1955). Various studies have conclusively demonstrated that *Capsicum* reduces the risk of developing atherosclerosis (hardening of the arteries) by reducing blood cholesterol and triglyceride levels (Kawada, 1986). Diabetic neuropathy is a painful nerve condition which can develop in cases of prolonged diabetes. Several double-blind studies have supported the considerable value of capsaicin creams for relieving the pain associated with this disorder (Tanden, *et al* 1992). Cayenne or *Capsicum* helps to stimulate circulation and has an energizing effect on the system. It has traditionally been used for overcoming fatigue and restoring stamina and vigor. It is considered a natural stimulant without the side effects of most stimulating agents (Elkins, 1996).

1.2.5 Objectives:

The information on relative specificity and recovery of mutation is a pre-requisite for practical mutation breeding. Mutagens have remarkable possibility of improving plants with regard to their qualitative and quantitative

characters. The investigations reported in the thesis have been carried out in M_1, M_2 and M_3 generations, keeping the following objectives in view;

- to study the effect of different mutagenic treatments on various biological parameters in M_1, M_2 and M_3 generations,
- to investigate the meiotic behaviour of chromosomes after mutagenic treatments in M_1, M_2 and M_3 generations,
- to find out the effectiveness and efficiency of mutagens in inducing mutations in M_2 generation,
- To induce maximum variations, with minimum damage of the plants, for the selection of mutants in M_2 and M_3 generations,
- to enhance the yield potential by isolating promising lines in M_2 and M_3 generations,
- to analyse the Capsaicin content of some of best promising lines by using HPLC technique.

Chapter-2

REVIEW OF LITERATURE

THESIS

REVIEW OF LITERATURE

The occurrence of sudden and heritable changes in the races was first suggested by De Vries (1901) in *Oenothera lamarckiana*. He proposed the use of radiation for the induction of mutations. The first successful use of X-rays was made by Muller (1927) for the discovery of induced mutability and its frequency in *Drosophila*. Later, the successes were achieved by Stadler (1929) in barley and Goodspeed (1929) in Datura and *Nicotiana*. The role of mutations in evolution was emphasized by Baur (1924) and Stubb and Wettstein (1941). Substitution and chromosomal reconstruction clearly demonstrated by Sears (1956) are now valuable tools in planned plant genetics and breeding.

Another phase in the history of induced mutations is the discovery of chemical mutagens during the World War II. So the use of chemical mutagens is comparatively recent in origin. Although the mutagenesis for the first time was tried by Schiemann (1912) by using potassium bichromate on *Aspergillus niger*, but the first successful attempt was made by *Auerbach & Robson (1942)* by using mustard gas on *Drosophila melanogaster*, whereas Oehlkers (1943) concentrated on the chromosomal translocations in *Oenothera* by urethane. Extensive work with chemical mutagens has begun only since 1960 following the introduction of EMS (Heslot, 1964). Rapoport (1947) studied the mutagenic effect of DES in Drosophila and postulated that ethylation is a mutation inducing process. Thereafter, DES has been largely used as a plant mutagen. In plants, the chlorophyll mutations with DES were first reported in barley (Heslot & Ferrary, 1958).

Alkylating agents are, by far, the most extensive and important groups of mutagens. However, only a few of the mutagens belonging to the group of alkylating agents such as, ethyl methane sulphonate (EMS), methyl methan sulphonate (MMS), diethyl sulphate (dES), ethyl imine (EI) and N-nitroso-N-methyl urea (NMU) have been reported to be most effective (Rapoport, 1962; Swaminathan, 1966; IAEA, 1970). In general, alkylating agents primarily induce chromatid type aberrations (Revell, 1953; Ockey, 1960; Kihlman, 1961; Evans & Scott, 1964; Heiner, 1971).Induced mutations are considered as

an alternative to naturally occurring genetic variations that serves as the source of germplasm for crop improvement programme and also as an alternative to hybridization and recombination in plant breeding. Mutagens have remarkable potential of improving plants with regard to their qualitative and quantitative characters; and where appropriate selection has been applied, improvement in yield (Brock, 1965., Gregory, 1968), adaptability (Gustaffson, 1965), maturity time (Brock, 1970) and numerous other traits (Sigurbjornson and Micke, 1969) have been reported. The extent to which induced mutation provide a useful alternative to the natural variation as a source of germplasm for the improvement of such trait is largely determined by the importance of linked group of genes and the degree to which natural selection has build up linked gene complexes of adaptive significance in the naturally occurring population (Brock, 1971).

3.1 Effect of mutagenic treatment on germination, survival, injury, sterility, seedling height, plant height:

There are many reports to demonstrate the effect of mutagenic treatments on germination, survival, injury, fertility, seedling height, plant height, yield and other morphological characters (Bhatacharjee *et al*., 1998; Khan, 1999; Mitra and Bhowmik, 1999; Khan & Wani, 2005; Jabee *et al*., 2008; Sharma & Anis, 1995; Omer *et al*., 2008; Jafri *et al*., 2011; Choudhary *et al* 2012). Increase in pollen sterility and decrease in seed germination with increasing doses of gamma rays in *Capsicum annuum* was reported by Rao and Laxmi (1980).

The effect of gamma rays and EMS on the seeds of *Capsicum annuum* L. has been studied by Asha and Nayar (1986) who observed an increase in Pollen sterility with increase in dose, and that gamma rays induced a higher percent of sterility as compared to EMS. The cytomorphology of the spontaneous triploid in *Capsicum annuum* L. showed delayed growth and prolonged flowering. It showed marked difference in fruit size and in the ratio of healthy and sterile seeds. (Chennaveeraiah and Habib, 1973).

Raghuvanshi and Joshi (1964) observed delayed and extended flowring with larger and varied number of floral parts in the colchiploids of *Capsicum frutescence*. Total sterility was observed in the fruits of *Capsicum annuum* produced in the radiation

induced polyploids (Indra and Abraham 1977). Pal *et al.* (1941) obtained fertile polyploid with larger fruits in the colchincine induced polyploids of *Capsicum annuum L.*

Lakshmi and Nalini (1989) isolated tertiary trisomic in *Capsicum annuum L.* (2n=24) and found phenotypic variations in the height, internodes and leaves. This trisomic had 2n=25 chromosomes. Sadanandam and Subhash (1985) isolated an aneuploid of *Capsicum annuum L.*, var. pusa jawala, followed by 40 kR gamma radiation. The variant was sufficiently vigorous in growth, dark green in colour.

Harini *et al.* (1990) obtained a chromosomal chimaeral plant with three distinct primary branches exhibiting diploids, mixoploids (diploid and tetraploids) and tetraploid condition. It was recorded for the first time in colchicine treated plants of X235, a local cultivar of chilli. These three branches showed differences in leaves, flowers, fruits and stomatal characteristics. The mixoploid branches displayed intermediate feature of both diploid and tetraploid branches. Further, fertility and yield were high in chimaeral plant as compared to those of diploid and tetraploid sibs.

Vandana and Dubey (1988) treated the seeds of *Vicia faba* L. with different concentrations of EMS and DES and found that germination, seedling growth, pollen fertility, time to maturity and survival were adversely affected by both the mutagens. Plant height, branching, number of leaves, pods and seeds as well as yield/plant showed varying responses to different concentrations of mutagens. However, DES at all doses and EMS only at the highest dose had adversely affected these traits whereas the lower dose of EMS had either no effect or a slight promoting effect.

Gautam *et al.* (1992) observed a direct relationship of pollen and ovule sterility with gamma rays and EMS doses in *Vigna mungo*, the maximum occurring at higher doses. Increase in pollen sterility and decrease in seed germination with increasing doses of gamma rays in *Capsicum annuum* was reported by Rao and Laxmi (1980).

Kumar *et al.* (1993) treated the seeds of *Vicia faba* L. with single and combined application of 0.75% DES and 10kR γ-rays. They recorded reduced germination, seedling growth, plant height, number of branches, number of pods, number of seeds/pod, test

weight, survival percentage and seed yield in the mutagenic treatments in addition to increased pollen sterility and delayed maturity. Application of γ-rays both singly and in combination with DES induced more severe effects than application of DES alone.

Bhatnagar (1984) reported the adverse effects of combined (EMS and Gamma rays) treatments on germination and survival of plants in chickpea. Reduction in seed germination with the increase in dose of gamma rays in chickpea was reported by Khanna (1991). The EMS treatment was found to cause higher sterility than gamma rays in chickpea (Kharkwal, 1981b).

Singh (2003) studied the effect of gamma rays, EMS and their combination treatments on germination and survival of plants in mungbean (*Vigna radiata* L. Wilczek) cultivars namely, T44 and PDM11. The germination and plant survival were obained highest at lowest doses of mutagens and combination treatments. The mutagenic effects were obtained higher at higher doses of both the mutagens individually, while in the combination treatments lower doses showed maximum effects. Varietal preference to the mutagens was also noticed.

Banu *et al.* (2004) made a comparative study in CO-6 and VBN-1 varieties of cowpea (*Vigna unguiculata* L. Walp) to study the mutagenic effects of gamma rays and EMS. Physical mutagen recorded higher percentage of reduction than chemical in M_1 generation. The mean values of all the characters taken for study i.e. germination, survival, plant height, seed fertility and pollen fertility, decreased as the doses of mutagens increased and there exist a linear relationship between them.

Dhamayanthi and Reddy (2002) treated the seeds of chilli (*Capsicum annuum* L.) var. Co-9 with gamma rays (10-35kR), EMS (0.5-1%), and MMS(0.5-1%) and studied the effect of these mutagens on seed germination, seedling survival, percent lethality and seedling injury. Lower doses were stimulative, while higher doses had inhibitory effect on these biological parameters. The highest percentage of seed germination and seedling survival were recorded at the lowest dose/concentration of all the mutagens used while the highest percentage of lethality and seedling injury were noticed at the higher dose/concentration of these mutagens. The stimulative effect on seed germination was

more in chemical treated plants than the physical mutagen. There was a proportionate decrease in germination percentage and seedling survival with an increase in concentration of both the chemicals.

Dose dependent decrease in these biological parameters has also been observed by Wani *et al.* (2004) in lentil and Kumar (2005) in *Coriandrum sativum* following treatments with EMS, and Kirtane and Dhumal (2004) in onion following treatments with SA, gamma rays and their combination. Mesharam *et al.* (1981) obtained a plant having spontaneous multiple translocation from normal populations of chilli cultivar CA-960. The plant was very healthy having broad green leaves and big size flowers.

Edwin and Reddy (1993) studied the effect of gamma rays, EMS and their combination treatments in hexaploid triticales. Reduction in germination, seedling survival and seedling height was observed in the treated populations. Combinations treatments were comparatively more effective followed by EMS treatments. An increase in chlorophyll variants, seedling injury, Pollen sterility, abnormal stomata were also observed in almost all mutagenic treatments.

Jayabalan and Rao (1987) studied the effect of gamma rays, ethyl methane sulphonate and nitroso methyle urea on the seeds of tomato cv, Co-2 and found decrease in percentage of seed germination and seedling survival with increasing dose/concentration. Reduction in germination, seedling growth and Pollen fertility following mutagenic treatments has also been reported in *Capsicum annuum L.* (Singh *et al.*, (1988).

Rangaiah *et al.*, (2002) studied the effect of gamma rays in hybrids (F_1M_1) and variety of chilli. A progressive reduction in seed germination, seedling growth and vigour in general with increasing doses was observed. The hybrid and varieties responded differently to gamma radiation, the former was less affected compared to the latter.

Seed germination and survival rate steadly decreased with increasing dose of two organophosphorous pesticides, Ekalux EC 25 and Metasystox on *Capsicum annuum L.* var. X235 and same result was shown by fungicide "Bavistin and Deltan" (Parakash *et al.*, 1988). X-ray treatment of chilli seeds at doses up to 15 kR resulted in reduced

seedling survival, an initial stimulating effect on growth, earlier flowering, morphological abnormalities and chlorophyll mutation. The highest dose also significantly increased pollen sterility (Sahib and Abraham, 1970).

Gradual reduction in germination and survival in *Capsicum annuum L.* was observed when treated with two insecticides "BHC and Nuvacron" and similar results were also obtained as shown by herbicides "Lasso and Basagran" treatment (Reddy and Rao, 1981 and 1982a). Jabeen and Mirza (2002) also reported similar results in *Capsicum annuum L.*

Laxmi and Gupta (1983) studied the response of different concentrations of EMS on various quantitative characters of *Trigonella* in M_2 generation. A significant gradual decrease in plant height, number of branches per plant, number of Pods per plant, number of grains per pod, pod length , and grain yield per plant was noted with an increase in EMS concentration.

Restaino (1983) isolated the pepper brachytic forms (*Capsicum annuum* L.) with different doses of ethyl methane sulphonate and gamma rays. Two recessive brachytic mutants were isolated from M_4, one was compact with reduced internodes, stems, and branches and other was semi compact with a slight reduction in length of the stem and internodes. Rajam *et al.* (1984) studied the effect of myomycin-C on soaked seeds of chilli. It reduced the percentage of seed germination and seedling survival drastically.

Rao *et al.* (1989) studied the effect of gamma rays, ethyl methane suphonate and nitroso methyle urea (NMU) singly and in combination on *Capsicum annuum L.* in M_2 generation and found that the mean values of most of the quantitative characters were lowered as compared to control and frequency of chlorophyll mutations increased following increased mutagenic treatments. Pollen sterility increased with an increase in dose of gamma rays. However, combination treatments were more effective than single doses.

Alkantara *et al.* (1996) determined the optimal conditions for mutagenesis in *Capsicum annuum* L. Seeds of cultivar Keystone resistant giant no. 3 were treated with

0.5%, 1.0% and 1.5% ethylmethane sulphonate (EMS) and exposed for 3, 6 and 9 hr. at 5°C , 10°C, 15°C, and 20°C. Several unique and interesting mutants were generated. In M_1 generation, seed treated with 1.5% EMS at 20°C for 9 hr. had the lowest germination percentage among 36 treatments, but they observed that differences in germination were not significant. They suggested that concentration and duration of seed exposure to EMS could be increased to induce even greater number of mutants.

Vandava and Dube (1988) observed reduction in seedling height and pollen fertility in *Vicia faba* by DES and EMS treatments, whereas lower doses of EMS exhibited slight promoting effect for morphological characters in *Vicia faba*. EMS, MMS and SA also reduced the seedling height in *Vigna radiata* L. (Khan & Wani, 2005). *Solanum melongena* L. (eggplant) showed a linear reduction in seedling growth with increasing doses of chemical mutagens (Hussein & Siddiqui, 1997; Shahab *et al.*, 2007). Reduction in plant height by Gamma rays was observed *Capsicum annuum* (Omer *et al.*, 2008). Varshney and Siddiqui (1997) found dose dependent decrease in plant height in bread wheat by the mutagenic action of thiourea. Linear reduction in plant height was also observed in *Oryza sativa* (rice) after the exposure of low UV-B radiations (Mohammed *et al.*, 2007). UV-B radiations reduced vegetative tiller production (25%) and total panicle dry weight (15%) in rice. A plant showing dwarf stature was identified from the progenies of 30 Gy gamma rays exposed population in *Hevea brasiliensis* (Saraswathy Amma *et al.*, 1990), whereas Trigonella foenum-graecum showed significant increase for plant height after gamma rays treatment (Yadav *et al.*, 2000). Caffeine showed a stimulatory effect on plant height and yield attributing characters at lower doses in *Capsicum annuum* L., while higher doses were found inhibitory (Kumar & Tripathi, 2004).

Reduction in plant survival and pollen fertility with increasing doses of gamma rays was reported in *Vigna mungo* (Sharma *et al.*, 2005), *Helianthus annuus* L. (Khursheed *et al.*, 2008). Survival and root length was inversely affected by increasing doses of gamma rays in *Capsicum annuum* L. (Omar *et al.*, 2008).

Capsicum annuum L. showed gradual decrease in seedling growth and pollen fertility in MMS treated populations (Sharma & Anis, 1995), whereas lower doses of MMS enhanced the percentage of germination, survival and pollen fertility in *Vigna radiata* L. (Khan *et al.*, 1987). Ethyl methane sulphonate (EMS) and its combinations with gamma rays have induced the seedling growth, pollen fertility and days to maturity in two varieties of *Lathyrus sativus* L. (Kumar & Dubey, 1998b)

Two varieties of *Capsicum annuum* L. namely CO-1 and Ujwala treated with different concentrations EMS showed a gradual increase in pollen sterility with increasing concentration of EMS. Percentage of sterility was recorded higher in variety Jawala than CO-1 (Salam and Thoppil, 2010). The effect of gamma rays and EMS was studied on *Jatropha curcus* L. The germination of treated plants had shown a sharp dose rate relationship, which decreased with increase in the dose/concentration of the mutagenic treatments. A decreasing trend in shoot and root length, seedling survival and vigour index with increasing dose/concentration of the mutagens has also been observed. EMS was more drastic in reducing seedling vigour than gamma rays. Lower doses of gamma rays has shown stimulatory effect on plant height whereas EMS treatments in both lower and higher concentrations showed inhibitory effect as compared to the control (Dhakshanamoorthy *et al.*, 2010).

Mutagenic potential of lead nitrate has also been studied on *Trigonella foenum graecum* L. Seed germination and pollen fertility decreased with increasing concentration of lead nitrate. Lower concentration showed stimulatory effect on plant height and number oof pods/plant. However, higher concentration showed inhibitory effect on these two parameters (Chaudhary *et al.*, 2012). Seed germination, pollen fertility and seedling survival showed a dose dependent reduction in *Vicia faba* L. after the treatment of DES and SA. However, DES showed inhibitory effect than SA (Bhat *et al.*, 2007). A reduction in seed germination and seedling survival with increasing dose of gamma rays in faba bean has also been observed (Mabrauk *et al.*, 2012). Reduction in pollen fertility with increasing doses of gamma rays was reported in safflower (Srivastava., 2012). Seeds *of Capsicum annuum* L. var. Azad treated with different concentrations of lead nitrate

showed a decreasing trend in seed germination, plant height and pollen fertility with increasing concentrations(Gupta and Kumar., 2008), and when the same var. treated with .5% EMS for 3, 5 and 7hr duration, the morphological parameter namely plant height, leaf area, no. of nodes and 100 seed weight showed a decreasing trend with increasing duration of treatment, however, days to maturity increased with the increase of the treatment hour (Kumar and Gupta., 2009).

3.2 Mutations Affecting Plant Morphology:

The availability of ample genetic variability is pre-requisite for attempting selection in plant breeding to develop desired plant types in any crop. Several induced morphological mutations have been reported in literature showing alterations in the morphology of various plant parts.

Singh (1988a) isolated 25 types of morphological mutants for plant habit, stem, leaf, height, flower and seed characters in chickpea. Generally, physical mutagens induce more morphological mutations and chemical mutagens induce more chlorophyll mutations (Gaul, 1960; 1964). Contrary to this, Singh 1988a) observed that EMS induced marginally more morphological mutations than gamma rays. Pleiotropic effect of morphological mutations was reported by Deshmukh *et al.* (1972). According to Blixt (1972) morphological changes are either as a result of pleiotropic gene action or of cryptic chromosomal deletions.

Variation in size, texture, type and modification of leaf parts have been reported by many workers (Patil, 1966; Venkatarajam and Subhash, 1986). Several workers have also reported mutants for plant height, maturity period, branching, seed and pod mutants (Raisinghani and Mahna, 1994 in *Vigna mungo*; Mary and Jayabalan, 1995 in *Sesamum indicum*). Singh *et al.* (1999) isolated several macromutations affecting different morphological character in *Vigna mungo* L. after treatments with gamma rays and EMS. Gamma rays induced bold seeded mutant was reported in broad bean (Bhat *et al.*, 2006a). The frequency of viable mutations has been found to increase with increase in the dose of EMS, NaN_3 and their combinations with gamma rays (Thakur and Sethi, 1995). Sharma (1970) reported synergistic effect for viable mutations at lower combination treatments as

against the additive effect observed at the higher doses. He further reported that the combination treatments changed the spectrum by inducing more mutation types that were not observed in the separate treatments. In chickpea, morphological mutants have been isolated for leaf shape (Kharkwal, 1981a), plant habit (Kharkwal, 1981b), growth habit, maturity, seed size (Vanniarajan *et al.*, 1993), Seed weight and total pods per plant (Khanna, 1981) and flowering period (Haq *et al.* 1989). Mutants have been isolated after seed treatment with physical and chemical mutagens. A wide range of mutants affecting habit, pod distribution, seed size and shape, earliness and resistance to *Ascochyta rabiei* were also obtained in chickpea due to seed treatment with gamma rays and EMS (Dekov and Radkov, 1982). Subhash *et al.* (1981) isolated Cluster bud mutants after treatment with EMS in *Capsicum annuum* L.

Gaikwad and Kotheker (2003) treated the seeds of two lentil cultivars, L-4611 and L-4639 with 3 different concentrations of 2 chemical mutagens, EMS and SA. Nine morphological mutants were isolated in M_2 and M_3 generations. These morphological mutants were named on the basis of the part of the plant body affected. Among them the early maturity, high yielding and bold seed type mutants have the potential to be incorporated into breeding programs.

Sharma and Kumar (2003) observed EMS induced macromutants in chickpea (*Cicer arietinum* L.) when treated the seeds with EMS (0.5%) at different durations. The morphological mutants were characterized on the basis of the part of the plant body affected. These mutants can be better fitted in new cropping patterns and with improved agronomic management, their yield ability may even be better. Gamma rays-induced semi-dwarf mutant with semi-dwarfing genes showed positive responses to salt stress in *Hordeum vulgare* (Forster, 2001).

Rajam and Subhash (1984) treated the seeds of *Capsicum annuum* L. with mytomycin-C for 30, 60, 90 and 120 min. Several morphological mutants affecting plant height, branching pattern, floral organs, fruit characters and other plant morphological characters were recorded from M_2 and M_3. Of special interest were the clustered bud, tall, multilocular, spindle fruit, erect fruit and orange fruit mutants.

Singh *et al.* (2004) treated the seeds of two improved cultivars of urd bean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 with single and combined doses/concentrations of gamma rays, EMS and SA. A number of various types of B morphological macromutations were induced in M_2 generation. Out of these, 14 mutants from PDU1 and 13 from T9 were identified as true breeding for plant morphology, pod and seed characters and early maturity in M_2 generation. Many macro mutants showed significant improvement in yield and other yield components as compared to their parents.

Sangsiri *et al.* (2005) detected gamma rays induced mutations in mung bean (*Vigna radiata* (L.) wilczek) when treated the seeds of two mung bean varieties and their F_1 and F_2 with gamma rays Cs-137 source) at the dose of 500 Gy. Mutant characters were grouped as chlorophyll, leaf, flower and pod mutants. Chlorophyll mutations included albino, coppery leaf, light green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaf, narrow-rugose leaf, multiple leaflet, round-cuneate leaf, unifoliate leaf, and wrinkled leaf. The flower mutant was cock's comb raceme while the pod mutant was a lobed one. All mutants were purified for genetic study and possible uses of the traits.

Solanki (2005) isolated twelve kinds of morphological mutations included changes for growth habit (compact, bushy, prostrate), foliage (narrow, broad, rogue, tendrillar), plant height (tall, dwarf), maturity and flowering behaviour (early, late, sterile) in lentil by EMS and SA treatments. The mutations for growth habit and foliage were induced with higher frequency by EMS, whereas those for plant height and maturity and flowering behaviour were induced with higher frequency by SA on the basis of sterility, SA was found more effective and efficient than EMS.

Kulthe and Kothekar (2006) observed induced morphological mutants in winged bean (*Psophocarpus tetragonolobus* (L.) DC.). The seeds of winged bean var. EC 38955 (A) were treated with three concentrations of two chemical mutagens, namely ethyl methane sulphonate (EMS) and nitrosomethyl urea (NMU). Various morphological mutants were recorded in M_2 and M_3 generations. These morphological mutants were named according to their morphology or special characters. Nine different mutants were isolated within

which high yielding, early maturing, dwarf, non-shattering pod mutants have useful potential and are indicative of the genetic improvement of winged bean crop Dhakshanamoorthy et. Al. (2010).

Kumar and Rao (2003) isolated six autotriploids from the progeny of an M_2 line of *Capsicum annum* L. var. PC1 whose progenitor was an M_1 semisterile (induced by 40kR gamma rays). These triplopids were characterized by large sized dark green leaves, stomata, pollen, flower and fruit growth besides longer petioles and greates plant spread. Nevertheless, these bear fewer branches, starter internodes, fewer fruits and seeds besides delayed growth and flowering compared to their M_1 progenitors and the control.

The frequency and spectrum of morphological mutations were found to be mostly dose dependent in *Capsicum annuum L.* (Kumar *et al.*, 2002). New cultivars having altered fatty acid composition have been released in gamma/X-rays irradiated rapeseed, sunflower, and linseed crops (Bhatia *et al.*, 1999). A light corolla mutant, showing variation in some of the oil constituents with a light purple eye at the base of flower was detected in gamma rays irradiated muskdana (*Abelmoschus moschatus*) [Mishra *et al.*, 2000].

Variation in carpel number and its morphology was observed in local brinjal cultivar treated with gamma rays and EMS (Zeerak, 1998). Two flower colour mutants, viz. red with white stripes and pink with white stripes were isolated in carnation through in vitro application of EMS (Singh, *et al.*, 2000a). The mutants not only performed better in the traits for which they were selected but also found to be important in other quantitative characters.

Gamma rays alone induced several micro and macro-morphological chimaeras in sunflower (Ratnam & Rao, 1994). Mini and deformed flower mutants were isolated in gamma rays irradiated *Beta vulgaris* L. (Chauhan *et al.*, 2006). The anthers in these mutants were both dehiscent and non-dehiscent, exhibiting a variable degree of pollen sterility associated with abnormal behaviour of endothecium and tapetum. Two dwarf mutants and one mutant with yellow pericarp were isolated from heavy ion irradiated sweet pepper (Honda *et al.* 2006).

Morphological mutants were observed in M2 generation of Black gram (*Vigna Mungo* (L.) Hepper)with the effect of dose/concentration of mutagens (EMS and Gamma rays) and such mutants were, dwarf, tall, tiny leaves, hairy leaves, male sterility, brown seed, early, maturing, long pod, bottom branching, top branching, bushy type, trailing and spreading habit mutants. EMS provided more number of morphological mutants followed by gamma rays (Arulbalachandran and Mullainathan, 2009).A dwarf mutant plant having small leaves and light-orange flower was identified in the M2 generation from gamma ray treated groundnut cultivar VRI 2 (Mothilal and Jayaramachandran. 2012).

Seeds of *Capsicum annuum* L. var. PC1 were exposed to five doses of gamma rays (10, 20, 30, 40 and 50 KR). Sixteen morphological mutant were isolated in M2 and M3 generations. These mutants were classified under four categories i.e. plant height mutant, leaf mutant, maturity mutant and fruit mutant (Kumar *et al*,. 2001). Tall, small broad fruit, dwarf, erect fruit, small pointed fruit, orange long fruit, orange round fruit, purple fruits mutant were isolated in *Capsicum annuum* L. cv. NP 46A, after the treatment with DMS, EMS, MES, HZ, HA and gamma rays (Raghuvanshi and Singh 1982).

The seeds of 2 varieties of chickpea, Pusa-212 and Pusa-372, were treated with gamma rays EMS and their combination treatments. The M2 population was carefully screened for various viable macromutations. A wide spectrum of viable morphological mutations affecting almost all parts of the plant were isolated in the M2 generation. The most striking mutants isolated included tall, dwarf, broad-leaved, white-flowered, bold-seeded and high-yielding mutants. Differences in varietal response to different mutagens was clearly evident as both of the varieties differed in the spectrum and frequency of mutations induced. Combination treatments were most effective in inducing a wider spectrum and maximum frequency of macro mutations, followed by EMS. Of all the morphological mutations, the frequency of leaf mutations was maximum, followed by plant height and seed mutants. Most of the macro mutants were confirmed to be true-breeding in the M3 generation except for the highly sterile ones. Some mutants were specifically isolated in a particular mutagen type (Wani, 2011).

3.3 Mutations Affecting Yield:

Medium or moderate doses of gamma rays under dry treatment and higher doses under soaked treatment were found more effective in inducing genetic variability for grain yield and its attributing characters in *Vigna mungo* (L.) Hepper (Kumar & Mishra, 2006). Gamma rays, ethyl methane sulphonate (EMS), mitomycin C (MC) and hydroxylamine (HA) induced a proportional increase in the female flowers in *Momordica charantia* L., resulting in a slight shift in sex ratio and yield (Mallaiah & Jafer, 1988).

Thirteen mutants with promising performance for yield components were isolated in M2 generation of gamma rays irradiated *Brassica juncea* Coss. (Javed *et al.*, 2000). Two mutants with significant increase in dry leaf yield per unit area were isolated in gamma rays irradiated *Virginian tobacco* (Ibrahim *et al.*, 2001b). A promising mutant (early maturity and high seed and husk yield) was obtained by combined treatment of gamma rays and ethidium bromide (EB) in *Plantago ovata* (Lal & Sharma, 2002). Several mutants with promising performance for yield and yield components have also been isolated in gamma rays, SA and EMS treated *Ocimum sanctum* L. (Nasare & Choudhary, 2003).

DES, EMS and colchicine increased the fruit yield in *Solanum melongena* L., and DES was found to be more effective in increasing fruit yield (Siddiqui *et al.*, 1988). Remarkable increase in fruit size, weight and number of fruits per plant was found in DES and MMS treated *Solanum melongena* L. (Siddiqui, 1989). NMU induced an increase in mean values for several quantitative characters in *Solanum melongena* L. (Siddiqui, 1993). Successful development of useful mutants with improved early seed maturity, coupled with high seed yield, seed quality and determinate growth habit in *Trogonella foenum-graecum* have been reported by EMS treatments (Basu *et al.*, 2007).

Loss in yield and its attributing characters was observed with increasing doses of gamma rays in different crops (Verma *et al.*, 1999; Pavadai & Dhanvel, 2005). Gamma rays have induced variability for various yield attributing characters in *Trigonella foenum-graecum* (Yadav *et al.*, 2000). Waghmare *et al.* (2001) isolated for the first time a fasciated mutant with less number of primary and secondary branches, reduced pod and seed size, low yield and delayed maturity in gamma rays irradiated *Lathyrus sativus* L.

seed size, low yield and delayed maturity in gamma rays irradiated *Lathyrus sativus* L. (grasspea). Reduction in root weight and shoot dry weight was observed with increasing doses of gamma rays in *Capsicum annuum* L. (Omar *et al.*, 2008). Gamma rays, EMS, streptomycin, acriflavin and ethidium bromide have reduced the biomass production in *Brassica juncea* (Singh *et al.*, 1993). Gamma rays and EMS showed a decreasing trend for the mean values with increasing dosage for five quantitative characters viz. primary branches per plant, clusters per plant, pods per plant, seeds and seed yield per plant in *Vigna unguiculata* (Banu *et al.*, 2005). Remarkable loss in yield has been experienced by chemical mutagens in soybean (Pavadai & Dhanvel, 2004) and bread wheat (Varshney & Siddiqui, 1997). *Gossypium hirsutum* L. (cotton) showed loss in plant height, no. of sympodia and no. of bolls per plant during M1 and M2 generations to the 13h EMS treatment (Sundaravadivelu *et al.*, 2006).

Three hexaploid triticales showed negative shift in mean for the plant height, tiller number and grain yield and positive shift in mean for spikelet/spike and 100-grain weight due to gamma rays and EMS treatments (Viswanathan *et al.*, 1994). Reduction in yield components was also observed in gamma rays irradiated caraway plants (*Carum carvi* L.), while induced increase in yield components for both *Foeniculum vulgare* (fennel) and *Nigella sativa* (black cumin) (Khalil, 2001).

Colchicine induced autotetraploids in faba bean (*Vicia faba* L.) showed gigantism, bigger leaves and flowers etc. with reduced pollen fertility, number of seeds per pod and number of seeds per plant as compared to diploids (Joshi & verma, 2004), whereas colchicine-induced autotetraploids have exhibited enhancement in yield attributing components in *Impatiens balsamina* L. (Dikshit & Kumar, 2007).

Four mutants with altered tannin content were screened in gamma rays irradiated winged bean (*Psophocarpus tetragonolobus* L. DC) (Klu *et al.*, 1997). Out of four mutants only one desirable mutant with a level of tannin of about 25% of the wild type and the other mutants having similar or increased tannin levels were recorded.

Ten agronomically desirable mutants were isolated in wheat and triticale after treating with gamma rays and EMS individually and in combination (Viswanathan & Reddy,

1998). Higher concentrations of EMS and its combination with gamma rays were found to be effective in increasing the variability for the fatty acid content in soybean oil (Patil *et al.*, 2007).

Cellulose synthesis was enhanced in green gram seedling by chelating agents viz. EDTA and 2, 2-dipyridyl at low concentration with a corresponding increase in amylase activity and a decrease in sugar content (Rao *et al.*, 1986).

3.4 Chlorophyll Mutations:

The chlorophyll mutation is the clear-cut indication of non directional nature of mutation and possibility of induction of useful mutations. Chlorophyll mutations are considered as one of the most dependable indices for evaluating the genetic effects of different mutagens in several crops (Gustaffson, 1951) and are used as genetic markers in basic and applied research (Reddy and Gupta, 1989). Different types of chlorophyll mutations such as albina, xantha, viridis, maculata, striata, chlorina etc. have reported in several crops by using physical and chemical mutagens (Swaminathan *et al.*, 1962; Reddy and Annadurai, 1991; Das and Kurdagrami, 2000, Singh *et al*., 2001). EMS has been reported to induce higher proportion of chlorophyll mutations than gamma rays in several crops (Waghmare & Mehra, 2001; Singh & Singh, 2001; Karthika & Lakshmi, 2006). The combined treatments of gamma rays and ethyl methane sulphonate (EMS) produced higher frequency and wider spectrum of chlorophyll mutations followed by single treatment of gamma rays or EMS in mungbean (*Vigna radiata* L.) (Singh *et al.*, 2005; Sharma *et al.*, 2006), while in *Vigna mungo* L. Hepper., the gamma rays was more efficient than EMS and their combination in producing chlorophyll mutations (Khan, 1999). Similarly gamma rays induced the higher frequency and wider spectrum of chlorophyll mutations than EMS in urdbean (Sharma *et al.*, 2005; Khan, 1999). Lower doses of gamma rays and EMS showed wider spectrum of chlorophyll mutations in *Nigella sativa* L. (Mitra & Bhowmik, 1999). Thus physical mutagen was found to be more effective in inducing chlorophyll mutations than chemical mutagen in two cultivars of soybean (Geetha & Vaidyanathan, 2000). Kumar *et al.* (2001) studied the frequency, spectra and inheritance pattern of chlorophyll mutations by gamma rays in a chili cultivar

X-235. Chlorophyll mutants were albino, chlorina, xantha and viridis. The frequency and spectrum of chlorophyll mutations by gamma rays have been found dose dependent in different crops (Palanivel & Jayabalan, 2000; Kumar *et al*., 2000; Jain *et al*., 2005). Dry and wet irradiated conditions influenced the rate of chlorophyll mutations in foxtail millet (Ichitani *et al*. 2003). A xantha mutant (yellow plant) was identified in gamma rays treated cytoplasmic male sterile (CMS) maintainer line 1132 of *Oryza sativa* L. (Zhou *et al*., 2006).

Prasad and Das (1980c) observed different types of chlorophyll mutations viz. albina, xantha, albo-xanthalba, alboviridis, virescence, chlorina, tigrina and maculata in six varieties of *Lathyrus sativus* L. The spectrum of chlorophyll mutations was found to be dependent on the genetic background of the genotype. Moreover, chlorophyll mutation frequency increased with the increase in dose of gamma rays both individually as well as in combination with MES in all the varieties. Contrary to this, Mitra and Bhowmik (1999) reported that lower doses of gamma rays and EMS showed wider spectrum of chlorophyll mutations in *Nigella sativa* L. Sharma (1970) reported that chlorophyll mutation frequency decreased at higher doses when calculated on segregating M_1 families basis. However, on the basis of M_2 plants a progressive increase with the increase in EMS doses was observed. Several workers have reported differential varietal response for the induction of chlorophyll mutation (Prasad and Das, 1980c; Singh *et al*., 1999; Das and Kundagrami, 2000). Sharma and Sharma (1981a) observed no varietal or mutagenic differences with regard to the spectrum and relative proportion of chlorophyll mutations.

Subhash and Venkat Rajam (1983) described the cytological and morphological variations induced in *Capsicum* cultivar C-5 after X-ray irradiation at 1, 3, 5 and 6kR doses. Chlorophyll mutants namely xantha, albina, straita and viridis were observed. The frequency of chlorophyll mutants increased as the doses of mutagen increased but at higher dose i.e. chlorophyll mutants did not observed .

Singh *et al*. (1999) observed the mutagenic effects of gamma rays and EMS alone or in combination on frequency and spectrum of chlorophyll and macromutations in two

cultivars, namely PDU, and T-9 of urdbean has been observed. Conclusively, the combination treatments have yielded the higher frequency and spectrum of chlorophyll mutations whereas the various doses of mutagenic agents have independent response towards macromutations in both the cultivars.

Yadav and Padmaja (2004) studied the induced chlorophyll mutations in the two varieties of *Cajanus cajan* (L) Millspaugh, viz. KPL 93115 and ICPL 93117, following the treatments of gamma rays and EMS. The chlorophyll mutants were quantified on the bases of M_2 seedlings and their frequencies were evaluated variety-wise and mutagen-wise for understanding mutagenic effectiveness of mutagens γ-rays and EMS used singly and in combination. Based on the frequency of chlorophyll mutants, ICPL 93117 variety appeared to be more responsive to γ-rays as well as EMS. Further, optimum results generated at 25 kR of γ-rays and 0.2% EMS indicated effectiveness of these doses.

Rajam *et al.* (1984) treated soaked seeds of Chilli with X-rays and 0.01% EMS separately and in combination. They observed six type of chlorophyll mutants viz., xantha , albina, chlorine, viridis and straita in M2 generation. Occurrence of chlorophyll mutations were in proportion to the dose duration and combined treatments enhanced the frequency of mutation.

Sharma *et al.* (2006) estimated the spectrum and frequency of chlorophyll mutation by using gamma rays, EMS and their combination on two cultivars, namely, Pant-19 and Pant-30 of urdbean (*Vigna mungo* L. Hepper). Five different types of chlorophyll mutations viz., albina, xantha, viridis, chlorina and maculata were identified in both the cultivars. Almost all the combination treatments produced maximum frequency and wider spectrum of chlorophyll mutations followed by single treatment of gamma rays or EMS. The frequency of chlorophyll mutations increased with higher doses of mutagens but decrease at highest dose.

Two varieties of *Trigonella foenum- graecum* L. viz., desi and kasuri methi were treated with different concentrations (0.1, 0.2 and 0.3%) of EMS, MMS and MES. A wide range of chlorophyll mutants were observed in both the varities. The mutants were albina, straita, xiathoalba and chlorina in both the varities. It was observed that the

chlorophyll spectrum with 0.3% MMS in desi methi. The highest chlorophyll mutation frequency was obtained with 0.3% EMS & chlorophyll mutation spectrum with MMS in kasuri methi, but the mutation spectrum was broader in desi methi as compared to kasuri methi (Vasu and Hasan., 2011).

The cultivar IPU-982 of Black gram was treated with different doses of gamma rays, sodium azide and their combined treatment. Six different types of chlorophyll mutants, namely, albino, xantha, dark xantha, chlorina, viridis and striata were induced. Out of these mutants, xantha and dark xantha were most frequent while striata was least frequent. The highest frequency of chlorophyll mutations (8.87%) was reported in the combination of 60kR+0.03%SA. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations whether mutagens were employed singly or in combination (Gaibriyal *et al.*, 2009).

Chilli var. K1 was treated with different doses/concentrations of gamma rays and EMS. Chlorophyll mutants such as albino, chlorine, viridis,virescens and lutescens were observed in M2 generation of treated seeds.Frequency and spectrum of chlorophyll mutations increased as irradiation and chemical mutagen doses increased. Generally, gamma rays induced higher proportion of chlorophyll mutants than EMS. *(Sri Devi and. Mullainathan., 2011).*

Dry and dormant seeds of *Capsicum annuum* cv. PC 1 were exposed to different doses of gamma radiation (5-50 krad) at 5 krad/minute, and subsequently sown in flower pots and then in the field (Andhra Pradesh, India). Six different chlorophyll-deficient mutant types (Albina, Xantha, Chlorina, viridis and Virescence) were isolated from the M2 and M3 progeny lines. The frequency and spectrum of the chlorophyll mutants were dose dependent and the Albina type predominated over the other types. All mutant types were recessive and controlled by a single gene. (Kumar *et al.*, 2000)

3.5 Mutagenic Effectiveness and Efficiency:

The usefulness of any mutagen (chemical or physical) in mutation breeding programmes depends not only on its effectiveness but also on its efficiency. Mutagenic

effectiveness is a measure of frequency of mutations induced by unit mutagen dose, whereas, mutagenic efficiency is the measure of proportion of mutations in relation to undesirable changes like lethality, injury, sterility, mitotic and meiotic chromosomal aberrations etc. In other words, the higher efficiency of a mutagen indicate relatively less biological damage. A highly effective mutagen may not necessarily show high efficiency and *vice versa*. Synergistic as well as antagonistic effects may occur when various physical and chemical mutagens are used in combination.

A distinction between effectiveness and efficiency of mutagenesis has been a major experimental activity in the past. The purpose of this exercise was to identify criteria by which efficient mutagen and mutagenic doses can be selected on the basis of analysis of M_1 parameters (Konzak *et al.*, 1965). Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens were studied in chickpea (Kharkwal, 1998a); *Oryza sativa* L. (Singh *et al.*, 2001); celery, fennel and ajowan (Paul & Datta, 2005). Lower doses of physical and chemical mutagens and their combinations were found to be effective and efficient in several crops by many workers (Prasad, 1972; Sharma & Sharma, 1981; Khan, 1999). It has been reported that among the monofunctional mutagens, methylating agents are more toxic and thus, need to be used only at lower concentrations (IAEA, 1970) as against ethylating agents that are reported to be less toxic and can be applied at relatively higher concentration to yield more mutations at equimolar concentrations.

With a view to enhance the mutation rate and also to alter the spectrum of mutations, many variations in treatment methodology have been used by different workers. Treatments with chemical mutagens have been given to dry as well as soaked seeds, seedling at different developmental stages, different phases of cell cycle at variable temperature and ionic concentrations (Chopra and Pai, 1979). Ramanna and Natrajan (1965) studied the mutagenic efficiency of certain alkylating agents under different treatment conditions of temperature and hydrogen ion (pH) concentration in barley. They

treatment conditions of temperature and hydrogen ion (pH) concentration in barley. They concluded that factors such as concentration and diffusion of the mutagen, rate of hydrolysis and the influence of alkylating and non-alkylating groups of the chemical play a considerable role in determining the mutagenecity of a compound.

According to some authors chemical mutagens have been reported to be more effective in causing mutations as compared to physical mutagens and to their combined treatments with physical mutagens (Raveendran & Jayabalan, 1997; Bhattacharjee *et al.*, 1998; Solanki & Sharma, 1999; Kharkwal, 1999, 2001; Shah *et al.*, 2006). MMS was found most effective and efficient than EMS in *Vigna radiate* L. (Wani & Khan, 2005), while EMS has been reported to be more effective and efficient than gamma rays in chickpea (Shah *et al.*, 2006), urdbean (Sharma *et al.*, 2005), *Lathyrus sativus* L. (Waghmare & Mehra, 2001), celery, fennel and ajowan (Paul and Datta, 2005). Sodium azide (SA) and gamma rays show higher effectiveness and efficiency in *Trigonella foenum-graecum* L. (Koli & Ramkrishna, 2002). Lower doses of hydrazine sulphate (HS) were found more effective and efficient, but followed a declining trend with increasing concentrations of HS (Jabee & Ansari, 2005).

The efficiency on the basis of seedling injury has been reported to be generally higher as compared with that based on pollen sterility. The efficiency of individual EMS and DES treatment was found 2 to 3 times higher in comparison to most other mutagenic treatments and the EMS proved itself to be more effective than DES (Kumar & Dubey, 1998b). Moreover the effectiveness and efficiency increased with increasing doses of gamma rays in sunflower (Ratnam & Rao, 1993). The combined treatment of gamma rays and EMS was found to be more effective than individual doses in generating the resistant type of mutants in Indian mustard (Yadav *et al.*, 2001). Similarly, EMS in higher concentrations as well as its combined treatments with gamma radiations was found to be more effective in inducing variability for the fatty acid content in soybean (Patil *et al.*, 2007). Khatod *et al.* (2002) reported that lower doses of gamma rays were found to be more effective in cotton.

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Ethylene imine has been reported to be more effective and efficient than gamma rays (Blixt, 1964). Higher mutagenic effectiveness of MMS was recorded in rice (Rao and Rao, 1983). Dixit and Dubey (1986) observed that NMU treatment was 2-5 times more efficient in comparison to gamma rays, whereas combined treatments showed higher efficiency than respective individual treatments. Higher efficiency of combination treatments has also been reported in barley (Khalatkar and Bhatia, 1975). Khan (1999) studied the effectiveness and efficiency of EMS, gamma rays and their combination in black gram. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens in chickpea has been reported by Kharkwal (1998a). Chemical mutagens have been found to be more efficient in inducing chlorophyll as well as viable and total number of mutations. NMU in particular was found not only to be effective but also efficient than gamma rays and EMS. Rao *et al.* (1991) observed that gamma rays were found to be more efficient than EMS in Chilli.

Kumar and Dubey (1998c) studied mutagenic effectiveness and efficiency of γ-rays, EMS, DES and their combination in *Lathyrus sativus* L. and reported an increase in injury with increasing radiation dose in individual as well as in combined treatments (γ-rays + EMS and γ-rays + DES). A substantial amount of sterility was induced in almost all treatments. The efficiency of individual EMS and DES treatments was 2 to 3 times higher in comparison to most other mutagenic treatments. EMS proved to be more effective than DES.

Banu *et al.* (2001) assessed the mutation frequency, effectiveness and efficiency of gamma rays and EMS in cowpea varieties (Co-6 and Vamban 1). Mutagenic effectiveness was higher at lower dosage and lower at higher dosage level. When comparing both the mutagens, EMS was found to be efficient in giving maximum mutations in Co-6, while in VBN 2, gamma ray treatments in general found to be efficient in providing more mutations.

Parveen *et al.* (2006) studied the efficiency and effectiveness of physical and chemical mutagens in inducing chlorophyll mutations in M_2 generation of *Trigonella foenum graecum* L. A comparative study of the frequency and spectrum of chlorophyll

mutations induced by MMS, EMS and gamma rays in M_2 generation was made in two varieties of *Trigonella foenum-graecum* viz. Paras-9018 and Krishna- 9001. Four different types of chlorophyll mutants viz., Albina, Xantha, Chlorina and Maculata were identified in the treated populations. Frequency of Xantha mutants was highest followed by chlorina and other types. Gamma rays in general proved to be more effective followed by EMS and MMS in inducing maximum frequency of chlorophyll mutations (gamma rays > EMS > MMS).

Kumar *et al.* (2012) Studied the mutagenic efficiency and effectiveness of gamma-rays (10, 15 and 20 kR) and ethyl methane sulfonate (EMS) (0.05, 0.1 and 0.2%) along with control in two varieties of Paprika cv. Bydagi Kaddi based on M_1 biological damages (lethality injury) and M_2 viable mutagen frequency. Mutagenic parameters like chlorophyll and total mutation frequency were also assessed in M_2 The results indicated variable response of the variety to gamma rays and EMS. EMS has been found more effective: while, gamma irradiations were found efficient for inducing viable mutation.

Shah *et al.* (2008) detected the comparative mutagenic effectiveness and efficiency of gamma rays and EMS in two desi (Pb2000 and C44), one Kabuli (Pb1) and one desi x kabuli introgression line (CH 40/91) of chickpea. The results revealed that EMS was almost seven times more effective and its efficiency was two times higher than that of gamma rays. Mutagenic effectiveness and efficiency were found to depend upon mutagen type and the genotype and both were higher at lower doses of EMS in three genotypes except in desi genotype C44. The introgression line desi X kabuli genotype was found to be most resistant towards mutagenic treatments than desi and kabuli types.

Mutagenic effectiveness and efficiency of gamma rays, EMS and combined treatments was studied in terms of lethality and chlorophyll mutations in two cultivars of soybean (Pusa-16 and PK-1042). In general the frequencies of chlorophyll mutations were high in gamma rays and combined treatments. Four types of mutants viz., albina, xantha, chlorine and viridis were observed in the study. Gamma rays were found to be more effective to induce chlorophyll mutations in both cultivars. PK-1042 cultivar exhibited

higher mutagenic efficiency as compared to Pusa-16 in EMS and gamma rays treatment. (Khan and Tyagi., 2010).

Dube *et all*., (2011) investigated the mutagenic efficiency and effectiveness of Gamma rays and EMS in alone and combination treatments in *Cyamopsis tetragonoloba* variety Sharada. The mutagenic efficiency and effectiveness in this plant was decreased with Gamma rays followed by EMS in combination treatments. The mutagenic efficiency recorded on the basis of percent lethality was more in all treatments as compared to it on the basis of percent injury. Gamma rays in alone treatments induced more mutagenic efficiency as compared to that of EMS in alone treatments or Gamma rays followed by EMS in combination treatments. However, the mutagenic effectiveness recorded was more in EMS alone treatments as compared to it in Gamma rays alone treatments or Gamma rays followed by EMS combination treatments.

The seeds of two varieties i.e., Pusa 212 and Pusa 372 of chickpea were treated with gamma rays, EMS and their combination treatments. Mutagenic effectiveness and efficiency was calculated based on biological damage in M1 and chlorophyll mutations in M2. Mutagenic effectiveness increased with the increase in dose/treatment. Combination treatment in general proved to be more effective followed by individual treatment of EMS and gamma rays. Mutagenic efficiency varied depending upon the criteria selected for its estimation. Intermediate treatments in general were found more efficient. The order of efficiency was gamma rays+EMS>EMS>gamma rays. Among the two varieties, var. Pusa 372 proved to be more sensitive to mutagenic treatments than the var. Pusa 212 (Wani., 2009).

Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments were studied in the genotype of cowpea variety CO-7. Gamma rays, EMS and combined mutagens produced a high frequency as well as a wide spectrum of mutation. The frequency of mutation was more in combined treatments than gamma rays and EMS. The mutagenic effectiveness and efficiency was calculated based on biological damage. In M1generation based on seed lethality and seedling injury and M2 generation was carefully screened for various chlorophyll and viable mutations. Mutagenic effectiveness

and efficiency increased with the decreased in dose or concentration. EMS was prooved to be more effective and efficient in causing mutations as compared to gamma rays and the combined treatments (*Girija and Dhanavel., 2009*).

Cowpea variety CO-6 was treated with EMS, DES and SA to assess the efficiency and effectiveness of these chemical mutagens. The mutagenic effectiveness was found to be the highest at lower concentration with all the mutagenic treatments. EMS was found to be more effective than DES and SA. Mutagenic efficiency varied depending upon the criteria selected for its estimation. Mutagenic effectiveness and efficiency decreased with increase in all mutagenic treatments (Dhanavel *et al*., 2008).

3.6 Induction of Cytological Aberrations:

Cytological studies during mitosis and meiosis are one of the most dependable index to obtain information regarding the role and the effect of the mutagens. It also provides considerable clue to assess radio-sensitivity of plants to both physical and chemical mutagens. Mutagen induced chromosomal aberrations have been reported by many workers in different plants such as Pea (Kallo, 1972), lentil (Reddy and Annadurai, 1992), fenugreek (Anis and Wani, 1997), *Capsicum annuum* (Anis *et al*., 2000) and broad bean (Bhat, *et al*., 2007a). Most of these workers observed dose dependent increase in the frequency of chromosomal abnormalities with respect to mutagenic treatments. Ignacimutu and Sakthivel (1989) observed a significant and positive correlation between chromosomal abnormalities and pollen sterility.

Rao and Laxmi (1980) studied the meiotic abnormalities induced by gamma rays in *Capsicum annuum L.* The meiotic abnormalities included stickiness, clumping of chromosomes, univalent multivalents, unequal separations laggard, and non orientation of chromosomes.

Rao and Kumar (1983) isolated three desynaptic mutants in a population of local cultivar of chilli. The mutant plant showed reduced chiasma frequency and pollen fertility than normal plants. The desynaptic plants were weak and medium strong types. The mutants showed a monogenic pattern of inheritance.

Sadanandam and Subhash (1984) studied the effect of EMS, DES and SA on chiasma frequency per bivalent and per pollen mother cell of M_1 plants in *Capsicum annuum L.* and observed a reduction in chiasma frequency in all mutagenic treatments compared to their respective control. EMS caused greater reduction in chiasma frequency per cell.

Meshram and Patil (1986) studied the cytological effects of dimethyl sulphate (DMS), ethyl methan sulphonate (EMS) and acid juice of mango (AJM) in *Capsicum annuum* L. and reported chromosome stickiness, univalents, multivalents, chromatin bridges, fragments and micronuclei.

Prakash *et al.*, (1988) studied the effect of two fungicides (Bavistin and Deltan) on *Capsicum annuum* L. var-X235 and reported a dose dependent increase in various chromosomal abnormalities namely univalents , multivalents stickiness, non-orientation of chromosomes, laggards and chromatin bridges. The mean chiasma frequency decreased with increased concentration of mutagens. A decrease in pollen fertility was also recorded with increased concentration of mutagen.

Anis and Sharma (1997) made cytological analysis in treated as well as in control plants of *Capsicum annuum* L. treated with EMS, MMS and SA and observed a reduction in chiasma frequency in all mutagenic treatments as compared to their respective control. EMS caused a greater reduction in chiasma frequency than MMS and SA. Various chromosomal aberrations like clumping and stickiness of chromosomes, univalents, multivalents and fragments were observed at metaphase–I.Irregualr grouping of chromosomes and laggards were also found at anaphase stages.

Anis *et al.*, (1998) induced autotetraplody in *Capsicum frutescens* var. suryamukhi by treating apical growing point with colchicine and observed various meiotic abnormalities such as univalents, multivalents, unequal distribution of chromosomes and micronuclei. These irregularities were the major factor for high sterility of pollen grains in induced tetraploid plants of *Capsicum frutescens*. Kumar and Dubey (1998c) studied the effect of gamma rays, EMS and DES on meiosis, pollen and seed sterility and survival percentage in M_1 generation of *Lathyrus sativus*. High frequency of translocations leading to

multivalent associations involving varied number nf chromosomes were induced in all the treatments.

Anis *et al.*, (2000) studied the effects of EMS, MMS and SA on various cytological parameter in M_2 generation of *Capsicum annuum* L. and recorded a greater reduction of chiasma frequency caused by EMS. Various meiotic abnormalities in M_2 plants such as univalents, multivalents, fragments, bridges and laggards were recorded. Pollen sterility was found to be increased with increase in concentrations of mutagens.

Kumar and Rao (2003) isolated six autotriploids from the progeny of a M_2 line of *Capsicum annuum* L. Autotriploid showed gigantism in respect of leaf stomata, flower and pollen sizes. However, they had fewer number nf flowers, branches, fruits and seeds, besides late flowering as compared to their M_1 progenitor and the cnntrol. Univalents, multivalents, unequal separation, micronuclei were the frequent chromosomal anomalies. The mean chiasma frequency, pollen fertility, seed fertility was lower as compared to M_1 progenitor and their control.

Kumar and Rao (2006) isolated fasciated stem mutant in a local cultivar of *Capsicum annuum* L. It was characterized by broad-strap like stem, increased plant height, days to maturity, and pollen sterility. Desyncpsis, nondisjunction of chromoshomes, chromosome clumping and stickiness, laggard and bridges were found in some of the PMCs of the mutant while the normal plant did not show these irregularities.

Kumar and Gupta (2009) induced karyomorphological variations in three Phenodeviants of *Capsicum annuum* L. Seeds were treated with 0.5% solution of EMS for 3, 5, and 7 h durations and genetic segregation was closely observed. Many chromosomal anomalies like stickiness, bridges, and multivalents, secondary associations, laggards, and precocious movement were observed in all the 3 durations of treatment. These anomalies showed a dose dependent increase in frequency. The morphological parameters showed a decreasing trend along with the increasing doses of treatments. However, with the 7 h dose 3 morphological variants were isolated which varied in plant height, number of nodes, leaf area, 100-seed weight (g) vigorousness and days to maturity, from other sib plants and also from control plants.

Subhash and Nizam (1977) reported that increasing dose of X-rays resulted into the formation of increased number of multivalents, fragments, bridges and micronuclei in *Capsicum annuum* L. Subhash and Venkat Rajam (1983) described the cytological and morphological variations induced in *Capsicum* cultivar C-5 after X-ray irradiation at 1, 3, 5 and 6kR doses. Gross chromosomal anomalies like fragments, bridges, laggards, unequal separation of chromosomes, micronuclei etc were observed at different stages of meiosis.

Katiyar (1978) studied *Capsicum annum* L. plants grown from gamma irradiated and control seeds for meiotic aberrations and pollen sterility in M_1 and M_2 generations. Chromosomal aberrations included stickiness, altered association, breakage, bridges, unequal segregation, laggards and abnormal microspores and their frequencies were dose-dependent. Pollen sterility showed dose dependent increase. The percentage of chromosomal aberrations were more in M_1 than M_2, which could be due to the operation of recovery mechanisms or elimination of damaged chromosomes in the intervening period. Similar results were also reported by (Rao and Laxmi 1980; Tarar and Dnyansagar, 1980)

Kumar and Rao (1985) isolated one desynaptic plant in a population of *Capsicum frutescens*. Meiotic studies in the desynaptic plant showed reduced chiasma frequency and pollen fertility. Chromosome pairing at pachytene was normal and complete in the control plant, while it was partial in the desynaptic. Twelve bivalents were regularly formed both at diakinesis and metaphase-I in the control plants, while univalents ranging from 12-24 were recorded at the corresponding stages in the desynaptic. At anaphase-I the chromosome segregation was regular (12:12) in the control and it was irregular in the desynaptic. It is presumed that desynapsis in *Capscium frutescens* may have been due to a spontaneous gene mutation.

Jayaabalan and Rao (1987) irradiated healthy, dry seeds of pusa ruby variety of *Lycopersicon esculentum* Mill. with gamma rays at 10 kR, 20kR, 30kR, 40kR and 50 kR dose levels. Meiotic studies were made in treated as well as in control plants. At metaphase I, meiotic abnormalities like clumping and stickiness of chromosomes,

univalents, multivalents fragments and irregular grouping of chromosomes were observed. At anaphase I, there were laggards and unequal grouping of chromosomes at poles.

Lakshmi *et al.* (1989) recorded cytomixis, between adjacent PMCs in a sterile plant screened in the population of Sindhur variety of *Capsicum annum* L. In 36.5% of pollen mother cells cytomixis was affected through cytoplasmic bridges resulting in PMCs with variable number of chromosomes ranging from 4-36. Interestingly, the phenomenon of cytomixis was associated with medium strong type of desynapsis. It was also observed that cytomixis has some sort of negative effect on desynapsis resulting in increased pairing in the cells involved in cytomixis.

Nirmala and Kaul (1993) detected desynaptic mutant in DES induced *Pisum sativum* variety Arkel, involving lack or impaired synaptic pairing, confined only to the male sex. This anomaly is controlled by a single nuclear recessive gene msg4, non-allelic to the other msg genes isolated in *Pisum sativum* genome. The synaptic anomaly leads to abnormal male meiosis involving premature chiasmata terminalization, nucleolar multiplication, univalency, unequal and irregular chromosome disjunction at AI and AII, unequal triads and tetrads and coenocyte formations. This resulted in degenerated microspore formation rendering the mutant total male sterile. The meiotic anomalies exhibited high proportion of variance and the initial anomalies add to the variance of the subsequent abnormalities making male meiosis exceedingly erratic. The major meiotic anomalies are inter-correlated but only some exhibit genetic correlations which unravel the causes and consequences of meiotic anomalies detected in this mutant. The dys gene causing the male sex specific anomalies, does not belong to the gene system, regulating chiasma formation and its terminalization in *Pisum sativum*. Instead, it is a special gene disrupting male meiosis only and is anther specific. Kumar and Rao (2003) isolated six autotriploids from the progeny of an M_2 line of *Capsicum annum* L. var. PC1and the mean chiasma frequencies in the triploids was significantly less than 1.5 times to that of their M_1 progenitor and their control.

Bhat *et al.* (2005a) provided a relative account of cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in *Vicia faba* L. Studies undertaken in M_1 generation on the variety minor of this species showed that both the physical and chemical mutagens induced various kinds of chromosomal aberrations and reduction in pollen fertility. Such effects were dose dependent and positively correlated with dose/concentration. However, the induction of meiotic aberrations was observed to be higher in MMS treatments followed by gamma rays and EMS, suggesting that MMS could be more effective in inducing genetic variability followed by gamma rays and EMS in this crop.

Bhat *et al.* (2005b) studied the relative effects of EMS and MMS on meiosis and pollen sterility in *Vicia faba* L. var. major in M_1 generation. Meiotic studies revealed various aberrations like stickiness, laggards, bridges, precocious separations, disturbed polarity, cytomixis and non-synchronisation. Stickiness of chromosomes was the most common aberration followed by bridges and precocious separation. Among the different stages of meiosis the frequency of chromosomal aberrations was maximum at metaphase-I stage and showed a linear increase with increase in concentration of both the mutagens. However, MMS induced maximum frequency of aberrations than EMS. Pollen sterility was the cumulative result of various meiotic aberrations.

Singh and Chaudhary (2005) observed γ-ray induced chromosome in two morphologically distinct varieties off *Capsicum annuum* L. When dormant, dry seeds of two varieties i.e. solitary pendent variety (LCA-335) and a clustering erect variety (RHRC-CE were irradiated with gamma rays. He reported that radiation induced meiotic abnormalities are directly proportional to the γ-ray doses administered). Altered association and other chromosomal aberrations included stickiness, clumping, bridges, laggards etc. Concomitantly, dose dependent increase in the percent pollen sterility ensued was directly proportional to the meiotic abnormalities. The percent frequency of genetic recovery or elimination of defectives in M_2 generation was greater than M_1. The pendent variety is genetically more stable than the clustering erect variety.

Bhat *et al.* (2006b) reported cytomixis during microsporogenesis in various stages of meiosis in MMS treated populations of *Vicia faba* L. Cytomixis was observed to occur through various methods, i.e. by forming cytoplasmic channels and direct fusion of pollen mother cells. The migration of nuclear content involved all the chromatin/chromosomes or part of it from donor to recipient cell/cells. The occurrence of PMCs with chromosome numbers deviating from diploid number (2n=12) through the process of cytomixis lead to the production of aneuploid cells in all the populations treated with various concentrations of MMS. Increasing concentration of MMS had a positive effect on the percentage of PMCs showing cytomixis. The level of pollen fertility was found to be affected by cytomixis and chromosome stickiness. It seems possible that genetic factors might have also contributed towards pollen sterility.

An aneuploid with the spectrum of anomalies including various associations of chromosomes at diakinesis, lagging chromosomes at anaphase, appearance of micronuclei at telophase–II and microspores with different constitutions of micronuclei was isolated in *Capsicum* by gamma rays (Sadanandam & Subash, 1985).

Gamma rays-induced six autotriploids were isolated in *Capsicum annuum* L. The triploids showed reduced chiasma frequencies and were characterized by large sized dark green leaves, stomata, pollen grains, flowers and fruits, besides longer petiole and greater plant spread (Kumar & Rao, 2003).

Kumar and Verma (2011) treated the seeds of *Vigna unguiculata* (cowpea) with gamma rays and sodium azide. They observed chromosomal aberrations like unorientation, multivalents, laggards, bridges, micronuclei, stickiness and precocious movements etc. Chromosomal aberrations were found to be correlated with the concentrations of both the mutagens individually as well as in combination. The combined treatment proved to be more effective in inducing chromosomal aberrations and sterility as compared to individual treatment sets.

Bhat *et al.* (2007) studied the comparative analysis of meiotic aberrations induced by DES and SA in *Vicia faba* L. Stickiness, stray bivalents, univalents, multivalent, laggards, bridges, cytomixis, micronuclei, disturbed polarity were the main chromosomal

aberrations and these aberrations increased with the increase in concentrations of each mutagen. The DES was more effective than SA as it induced more chromosomal aberrations.

Seeds of *Capsicum annuum* L. varieties CO-1 and jwala were treated with potent chemical mutagen, Ethyl methane sulphonate. Various types of meiotic chromosomal aberrations such as multivalents, stickiness, clumping, bridges, laggards, micronuclei, tripolar orientation, pentad, non synchronous separation etc., and a dose dependant decrease in pollen fertility were observed in M1 generation. The frequencies of chromosomal abnormalities increased with the increase in mutagenic concentrations. Varietal response to the chromosomal aberrations was very pronounced, i.e. the variety jwala was more sensitive and the frequency of aberrations was comparatively high at all the mutagenic concentrations (Salam and Thoppil., 2010).

Seeds of *Capsicum annuum* L. var. G4 were subjected to different concentrations of methyl methane sulphonate (MMS) and diethyl sulphate (DES). Various types of meiotic aberrations such as univalents, multivalents, stickiness, bridges, laggards, cytomixis etc. were observed in all the treatments. However, the MMS treatments proved to be more effective in inducing meiotic aberrations as compared to DES. The frequency of meiotic aberrations was maximum at metaphase followed by anaphase and telophase stages. As the concentrations increased, reduction in chiasma frequency and pollen fertility was observed in all the treatments and, MMS again was found to be more effective than DES treatments (Gulfishan *et al.*, 2012).

In the light of above summarized literature, it may be concluded that a great deal of work has been done on the mutagenic properties of different mutagens in several plants. At present methyl methane sulphonate (MMS) and diethyl sulphate (DES) has been employed to assess their mutagenicity and cytogenetic assay in chilli (*Capsicum annuum* L.) in M_1, M_2 and M_3 generations to induce genetic/morphological variability for the selection of mutants which may be better than the existing strains.

Chapter-3

MATERIALS AND METHODS

MATERIALS AND METHODS

2. MATERIALS:

Capsicum annuum L. has been selected for the present experiment. The effect of chemical mutagens (MMS and DES) has been studied on cytomorphological characters of *Capsicum annuum* L.

2.1 Varieties Used:

Two commercial varieties of chilli (*Capsicum annuum* L.) viz. Pusa jwala and G4 were used for the study. A brief description of these two varieties is given below.

Table 3. Brief Description of the two varieties viz. Pusa jwala & G4 of *Capsicum annuum* L.

Variety	Procured from	Salient Features
Pusa jwala	**IARI New Delhi**	Plants 50-60 cm tall erect, fruits thin, red; 2-4 fruits of early flush are erect to semi-erect and subsequent are pendent on the same plant, Adaptable throughout India, mature up to 115-125 days, highly pungent, high yielding under irrigated conditions, most popular virus resistant variety. Chromosome number 24.
G4	**IARI New Delhi**	Plants average height 53 cm. Fruits olive green turning red, thin pendent pointed tip, mature upto 175-185 days, Adaptable throughout India, less pungent than Pusa jwala. Resistant to bacterial leaf spot but susceptible to root knot nematode. Chromosome number 24.

2.2. Mutagens Used:

The following two mutagens were used separately. The concentrations of each mutagen used in the present study are given below (Table 4).

2.2.1 Methyl Methane Sulphonate (MMS), [($C_2H_6O_3S$)].

The alkylating agents have been found to be the most potent in a wide array of organisms. Within the alkylating groups, MMS has been found to be a very effective chemical mutagen. Like other alkylating agents, MMS reacts with DNA by alkylating the phosphate groups as well as purine and pyrimidine bases and create a gap between DNA molecule causing mutation. It is a colourless liquid with a molecular weight of 110.3.

2.2.2 Diethyl sulphate (DES), [$(C_2H_5)_2SO_4$].

It is also an alkylating agent. Rapoport (1947) studied the mutagenic effect of DES in Drosophila and postulated that ethylation is a mutation inducing process. There after DES has been largely used as a plant mutagen. It reacts with DNA bases, predominantly with the N-7 of guanine but also with the N-1, N-3 and N-7 of adenine and the N-1 and N-3 of cytosine (Singer and Fraenkel-Conrat 1975). Besides having two alkylating groups, it acts as monofunctional agents, since each group alkylates separately. It is highly toxic and suspected carcinogenic agent with a molecular weight of 154.20.

2.2.3 Preparation of Mutagenic Solutions:

One percent stock solutions of methyl methane sulphonate (MMS), dimethyl sulphate (DMS) and diethyl sulphate (DES) were prepared and then different concentrations were prepared by using the following formula:

$$S1\ V1 = S2\ V2$$

Where:

S1 = Strength of stock solution

V1 = Volume of stock solution

S2 = Strength of desired solution

V2 = Volume of desired solution

The specificity of the action of chemical mutagen depends upon particular conditions of treatment, the more important of which are temperature and hydrogen ion concentration of mutagenic solution. During the course of present study, solutions

of MMS, DMS and DES were prepared by dissolving appropriate quantities of these chemicals in phosphate buffer having a pH 7.0 and the final pH adjusted to 7.0 by adding few drops of normal NaOH/HCl with the help of Backman's pH meter.

Table 4. Details of MMS and DES treatments given to chilli seeds of var. Pusa jwala & G4.

Mutagen	Conc.	Duration of	Duration of	pH	No. of seeds
Used		Presoaking (h)	Treatment (h)		treated
Control	-	12 h	-	-	300
	0.01%	12 h	6 h	7.0	300
	0.02%	12 h	6 h	7.0	300
MMS	0.03%	12 h	6 h	7.0	300
	0.04%	12 h	6 h	7.0	300
	0.05%	12 h	6 h	7.0	300
	0.01%	12 h	6 h	7.0	300
	0.02%	12 h	6 h	7.0	300
DES	0.03%	12 h	6 h	7.0	300
	0.04%	12 h	6 h	7.0	300
	0.05%	12 h	6 h	7.0	300

2.2.4 Method of Treatment with Chemical Mutagens:

Prior to the mutagenic treatment the seeds were presoaked in distilled water for 12 hours at room temperature (25±1). After the completion of presoaking period the seeds were kept on blotting paper so as to remove small droplets of water adhering to the surface of seeds. Thereafter the seeds were treated in different concentrations of chemical mutagens for 24 hours.

The control seeds were also soaked in distilled water but kept untreated for simultaneous physiological activities, as that of treated seeds.

During chemical mutagenic treatments the intermittent shaking was given throughout the treatment period to facilitate sufficient aeration and maintenance of uniform concentration of mutagen around the seeds. After the treatment period, the

treated seeds were thoroughly washed in running tap water before they were sown in earthen pots.

2.3 Sample Size:

In each variety a set of 200 seeds was used for each dose including the control. Out of these seeds, 150 seeds in each treatment were sown in earthen pots and then transplanted to field at 4 to 5 leaves stage for morphological and cytological studies, whereas the remaining set of 50 seeds was also sown in separate earthen pots for measuring root-shoot length (Seedling Height).

2.4 Sowing of Seeds:

The treated as well as untreated seeds were sown in 30 cm diameter earthen pots (50 seeds in each pot) for raising the seedlings. The seedlings at 4 to 5 leave stages were transplanted to well prepared experimental field in a complete randomized block designs (CRBD) in three replicates. Recommended agronomical practices were employed for the preparation of field, sowing and subsequent management of populations to raise a nice crop.

2.5 EVALUATION OF M1 GENERATION:

A detailed study of the effect of different mutagenic treatments in the two varieties was undertaken using the following parameters.

2.5.1 Seed Germination:

Germination data were recorded every alternate day upto 30 days after sowing, till the maximum germination was attained. The germination percentage based on number of seeds sown and germinated, was calculated by the following formula

$$\text{Germination percentage}(\%) = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Inhibition}(\%) = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

2.5.2 Seedling Height:

Seedling height was calculated on 12^{th} day of germination of seeds in pots by measuring root and shoot length of randomly selected seedlings from each treatment as well as control. Seedling injury was estimated by reduction in the root and shoot lengths, calculated in terms of percentage.

$$\text{Percent Injury}(\%) = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

2.5.3 Plant survival (%)

The Surviving plants in different treatments were counted at the time of maturity and following formula was used to calculate percent survival and percent lethality

$$\text{Survival (\%)} = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100$$

$$\text{Lethality(\%)} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

2.6 CYTOLOGICAL STUDIES

2.6.1 Meiotic abnormalities:

Cytological studies were carried out on pollen mother cells (PMCs) by fixing young flower buds from each treatment as well as control. The purpose of fixation is to kill the tissue without causing any distortion of the components to be studied. For meiotic studies, young flower buds from 40-45 randomly selected plants were fixed in freshly prepared Carnoy's fluid (alcohol : chloroform: acetic acid in 6:3:1 ratio) supplemented with crystals of ferric chloride for 24 hours. The material was then washed and preserved in 70% alcohol at 4°C. Meiosis was studied by squashing the anthers in 2% acetocarmine. The slides were made permanent by dehydrated them in n-butyl alcohol series (NBA series) (Bhaduri and Ghose, 1954) followed by mounting in canada balsam and then slides were kept in incubator at 45°C temperature till drying. After drying, the extra amount of canada balsam remained outside the cover slip was cleaned with xylene. Analysis of various stages of meiosis was done from each treatment as well as control at metaphase I/II, anaphase I/II and telophase I/II by studying more than 200 dividing PMCs. The abnormalities were recorded on the basis of variations in structure and behaviour of chromosomes as compared to control. The photomicrographs were taken from temporary as well as permanent slides with the aid of "Nicon", photomicrographic unit at the magnification of 1000 X (10 x eye piece X 100 x objective lens).

2.6.2 Pollen fertility (%)

Fresh anthers of randomly selected control and treated plants were squashed in 2% acetocarmine. The pollen grains which took the stain and had a regular outline were considered as fertile, while the empty ones without stain and having irregular

shape were considered as sterile. The percent fertility and percent reduction was calculated as follows:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

$$\text{Percent reduction (sterility) (\%)} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

2.6.3 Chiasma frequency:

The number of chiasmata per cell and per bivalent were estimated in treated as well as control plants by scoring 100 PMC at random at metaphase-I stages.

2.7 Frequency of Morphological Variations/Mutations:

The morphological variations/mutations were scored on the basis of characters in control plants and their deviations in the treated populations at older stage (90 days old). Following formula was adopted to calculate the frequency of variations/mutations.

Frequency of variations (%) =

$$\frac{\text{Number of varied plants at older stage } (M_1 \text{generation})}{\text{Total no. of germinated seedlings}} \times 100$$

Mutation Frequency (%) =

$$\frac{\text{Number of mutated plants at older stage } (M_2 \& M_3 \text{generation})}{\text{Total no. of germinated seedlings}} \times 100$$

2.8 Quantitative characters:

Following seven quantitative characters were statistically analyzed to assess the extent of induced variability in M_1 generation.

Days to flowering: Days to flowering were noted as the number of days taken by the plant from the date of sowing till the first flower appeared in the plant.

Plant height (cm): Plant height (cm) was measured at maturity from the base upto the apex of plant.

Days to maturity: Days to maturity were noted as the number of days taken by the plant from the date of sowing to the date of harvesting of the plant.

Number of fruits per plant: The average number of fruit per plant was determined at maturity.

Fruit length (cm): Twenty fruits were selected randomly from each selected plant and there length was measured and mean length of fruits was determined in each treated populations as well as control.

Fruit girth (cm): The girth at the mid position of the twenty randomly selected fruits from each selected plant was measured and the mean value of girth from each selected plant was calculated.

Total yield per plant (g): Total yield per plant was the weight of total number of fruits harvested from each plant and the yield of each plant was recorded in grams. Data related to these characters were taken from 30 randomly selected plants in control and treated populations and divided by the number of plants or observations to obtain the average in their respective units.

Selfing:

The selected variants were selfed in M_1 generation to induce homozygosity and for screening of the mutants in M_2 generation.

2.9 EVALUATION OF M2 GENERATION:

The collected seeds in M1 generation were harvested separately in treated as well as control populations. A set of control seeds and all those obtained from treated populations of M1 generation were sown for study in M2 generation. Three replicates were maintained in each treatment.

2.9.1 Mutagenic Effectiveness and Efficiency:

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagens, whereas, mutagenic efficiency is the measure of proportion of mutations in relation to undesirable changes like injury, lethality, sterility and meiotic aberrations etc. Mutagenic effectiveness and efficiency was calculated on the basis of formula suggested by Konzak et al (1965).

$$\text{Mutagenic Effectiveness} = \frac{\text{Mutation rate } (M_2 \text{ family or population basis) MP}}{\text{Concentration of mutagen (C)} \times \text{Duration of treatment in hours (T)}}$$

Percentage of mutated plant progenies (mutation rate in

$$\text{Mutagenic efficiency} = \frac{M_2)\ MP}{\%\ \text{injury (I) or \% lethality (L) or \% sterility(S) or \% meiotic aberrations (M)}}$$

2.9.2 Studies on Different Morphological Traits:

For morphological studies the parameters were similar to those in M1 generation. These include: Days to flowering, Days to maturity, plant height, No. of fruits, fruit length, fruit girth, and yield per plant.

2.10 Cytological Studies:

Cytological studies in M_2 progeny were carried out as in M_1 generation

.Selfing:

The selected mutants were selfed in M_2 generation also.

2.11 EVALUATION OF M3 GENERATION:

The plants in M_3 generation were raised from the seeds obtained from the selected mutants of M_2 generation and studied separately along with control population. The characters studied in M_3 generation were same as in M_2 generation.

2.12 STATISTICAL ANALYSIS

The data recorded on different characters due to mutagenic treatments have been subjected to statistical analysis with a view to find the individual and comparative effects of different mutagens. Mean, standard error, standard deviation and coefficient of variation were calculated as per the standard statistical procedures.

2.12.1 Mean ($\overline{X}$):

The mean value was computed by taking the sum of a number of observations and dividing it by the total number of observations recorded, thus

$$\overline{X} = \frac{x_1 + x_2 + \ldots\ldots x_n}{N}$$

$$\overline{X}\,(\text{Mean}) = \frac{\sum X_n}{N}$$

Where, x_1, x_2.....x_n=observations

N = Total number of observations involved

2.12.2 Standard deviation (S.D.):

Standard deviation is the positive square root of the average of sum of squares of deviations of all observations from their means. It is calculated by the following formula.

$$S.D.=\sqrt{\frac{(\bar{x}-x_1)^2+(\bar{x}-x_2)^2 \cdots (\bar{x}-x_n)^2}{n}}$$

$$S.D.=\sqrt{\frac{\sum(x-\bar{x})^2}{n}}$$

where,

Σx = sum of all individual observations

$\bar{x}$ = Mean of all observations

n = Number of observations

2.12.3 Standard Error (S.E.):

It is the measure of uncontrolled variation present in a sample. It is estimated by dividing the estimate of standard deviation by the square root of the total number of observations in the sample, Thus

$$S.E. = \frac{\text{S.D. of sample}}{\sqrt{N}}$$

Where,

S.D. = Standard deviation
N = Number of observations

2.12.4 Coefficient of variation (C.V.)

It measure the relative magnitude of variation present in the observations relative to magnitude of their arithmetic mean. It is defined as the ratio of standard deviation to arithmetic mean expressed as percentage and is a unit less number. It is computed by applying the following formula :

$$C.V.(\%) = \frac{S.D.}{\bar{X}} \times 100$$

Where,

S.D. = Standard deviation

$\bar{X}$ = Arithmetic mean

2.12.5 Least Significant Difference (LSD):

The least difference was applied and computed as follows:

Step 1: According to treatment given, construction of data table for treatment and 3 replicates.

The data were compiled in such a way that each treatment occupies a row and their replicates were arranged in column.

Column (Treatments)	Column (Replicates)			Total of Rows (Treatments)
	R_1	R_2	R_3	
T_1	A_1	A_2	A_3	$A_1+A_2+A_3=X_{r1}$
T_2	B_1	B_2	B_3	$B_1+B_2+B_3=X_{r2}$
T_3	C_1	C_2	C_3	$C_1+C_2+C_3=X_{r3}$
T_4	D_1	D_2	D_3	$D_1+D_2+D_3=X_{r4}$
T_5	E_1	E_2	E_3	$E_1+E_2+E_3=X_{r5}$
T_6	F_1	F_2	F_3	$F_1+F_2+F_3=X_{r6}$

Total of A_1 +.....F_1 = XC_1, A_2+.....F_2 = XC_2, A_3 +.....F_3 = XC_3 = Grant Total (G)

Step 2: Correction Factor (CF).

$CF = (G)^2 / T \times R$

Where:

T = Number of treatments

R = Number of replications

Step 3: Total Sum of Squares (TSS): This is the sum of square of all the observations minus correction factor

TSS = $[(A_1)^2 + (B_1)^2 + \ldots\ldots\ldots\ldots\ldots(F_3)^2] - CF$

Step 4: Replication Sum of Squares (RSS).

$$\textbf{RSS} = \frac{(XC_1)^2 + (XC_2)^2 + (XC_3)^2}{T} - CF$$

Where:

T = No. of treatments

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Step 5: Treatment Sum of Squares (TrSS).

$$\text{TrSS} = \frac{(Xr_1)^2 + (Xr_2)^2 + (Xr_3)^2}{R} - CF$$

Where: R = Number of replications

Step 6: Error Sum of Squares (ESS).

ESS = TSS – (RSS - TrSS)

Step 7: Construction of ANOVA table.

Source	Degree of Freedom	SS	MS	F. value
Replication	R-1	RSS	RSS / R-1 = RMS	TrMS/EMS
Treatment	T-1	TrSS	TrSS / T-1 = TrMS	
Error	(R-1) (T-1)	ESS	ESS / (R-1) (T-1) = EMs	

Step 8: Least significant difference based on ordinary test.

LSD at 5% level = $\sqrt{\frac{2EMS}{R}} \times$ (t value at 5% level)

LSD at 1% level = $\sqrt{\frac{2EMS}{R}} \times$ (t value at 1% level)

Where:

t = Tubulated value

If the difference between any two samples means exceeding the LSD value obtained at 5% level and / or 1% level, the difference between the two means is said to be significant at 5% or 1% level respectively.

2.13 CAPSAICIN ESTIMATION:

Capsaicin content of some of most promising isolated mutant lines in M_3 generation were analyzed. The selection of mutants from the isolated mutant lines in M_3 for capsaicin estimation was based on morphological/economic characters.

2.13.1 Preparation of Samples:

The harvested fruits were dried at 50° C in oven and grounded into fine powder and were kept in airtight containers at room temperature, prior to extraction. For

extraction of capsaicin, 1 gm finely grounded powdered sample of each mutant were dissolved in 10 ml of acetonitrile with two hr of shaking on a shaker. After that, flask was left overnight at room temperature. The supernatant was filtered with Whatman filter paper No.1, thereafter by 0.45μm filter paper and with a 10 ml disposable syringe into 1 ml glass vial. The volume of injection in HPLC was taken 10 μl. The standard solution of capsaicin (Sigma Chemicals Co. M2028) were prepared as 50 ppm stocks in absolute ethanol and used for retention time verification and instrument caliberation.

2.13.2 Estimation of Capsaicin by High-Performance Liquid Chromatography (HPLC):

The samples were analysed using Watre's Quaternary Gradient HPLC system equipped with Waters 717 autosampler, temperature controller, Water-966 photodiode aaray detector and Millennium[32] software for data processing. Reverse phase HPLC was carried out on a Spherisorb RP C-18 ODS-1 column(150mm×46mm), having particle size 5 μm. A pre-column guard cartridge Spherisorb RP C-18 were also used. The capsaicin was determined under uniform HPLC conditions: column temp. 30 °C, flow rate 1.5 ml/min. and run time was 20 minutes. The mobile phase was isocratic with solvent combination (Acetonitrile: water containing 1.00% acetic acid in 70:30) and the detection was at 280nm.

Chapter-4

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

4.1 STUDIES IN M_1 GENERATION:

The mutagenic effects of methyl methane sulphonate (MMS) and diethyl sulphate (DES) were evaluated on seed germination, plant survival, pollen fertility, seedling height, plant height, various quantitative characters in M_1 generation and meiosis of two varieties of Chilli (*Capsicum annuum* L.) viz., Pusa jwala and G4.

4.1.1 Seed Germination:

Seed germination was recorded from 10^{th} to 30^{th} days after sowing till the maximum germination was noted in control as well as treated seeds. Percentage of seed germination decreased with increasing concentrations of each mutagen in both the varieties. The maximum reduction in germination was observed at the maximum concentration of both the mutagens. In case of MMS treatments, the maximum inhibitory effect on seed germination (i.e., 46.02% and 42.18%) was observed at 0.05% in var. Pusa jwala and G4 respectively. In case of DES treatments the maximum inhibition was 44.45% and 40.25% at 0.05% in var. Pusa jwala and G4 respectively (Table 5 & 6). In general, germination was affected in all the treatments in both the varieties. However, var. Pusa jwala was more sensitive to the mutagenic treatments than var. G4. The order of effectiveness was MMS>DES, in both the varieties.

4.1.2 Plant survival:

The survival of plants in both the varieties decreased with an increase in concentration of both mutagenic treatments (Table 5 & 6). The maximum survival percentage was observed at the lowest treatments, while minimum survival percentage was recorded at highest concentration of both the mutagens in both the varieties. The maximum lethality i.e., 44.91% in var. Pusa jwala and 42.08% in var. G4 was recorded at 0.05% MMS treatments respectively. In DES treated population the maximum lethality was 42.10% and 37.12% in var. Pusa jwala and G4 respectively. The order of plant lethality was MMS>DES in both the varieties. However, var. Pusa jwala was more sensitive than var. G4 as the survival in Pusa jwala was more adversely affected by both the mutagens.

4.1.3 Pollen fertility:

Pollen fertility is also one of the important parameter in mutation breeding which was reduced with the increasing concentrations of both the mutagens. In other words, the pollen sterility increased with increase in mutagenic concentrations. The percentage of pollen fertility in control was 96.00% and 95.00% in Pusa jwala and G4 respectively, but decreased in all the treatments in M_1 generation from 84.25 to 66.60% and 83.10% to 70.19% in 0.01%-0.05% MMS treatments in var. Pusa jwala and G4 respectively, while in DES treatments it was reduced from 86.30%-69.70% and 85.32%-71.85% in var. Pusa jwala and G4 respectively (Table 5 & 6). The MMS was highly effective on pollen fertility as compared to DES in both the varieties.

Consequently the relative reduction in pollen fertility i.e., 30.62% was observed in 0.05% MMS followed by 27.39% in 0.50% DES in var. Pusa jwala, while in variety G4 the relative reduction was maximum i.e., 26.11% in 0.05% MMS followed by 24.36% in 0.05% DES (Table 5 & 6). It was seen that response of both the varieties was different with these two mutagens.

4.1.4 Seedling height (cm):

The seedling height (root length + shoot length) was measured and presented in Tables 7 & 8. The mean values of seedling height decreased with increasing concentrations of both mutagens in both varieties. The average height was reduced gradually from 10.36 cm in control to 5.30 cm in 0.05% MMS and 5.50 cm in 0.05% DES in var. Pusa jwala (Table 7), whereas, in var. G4 it was reduced to 5.02 cm in 0.05% MMS and 5.27 cm in 0.05% DES in comparison to control (i.e.,10.63cm). The maximum reduction in seedling height was recorded at the highest concentration of both the mutagens in both the varieties (Tables 7 & 8). The mean values of seedling height and percent injury indicated that MMS was more effective than DES.

4.1.5 Frequency of variations at older Stage:

The variations in control populations were absent. The frequency of variations such as Tall/dwarf, stunted/improved growth; improved/poor branching and fruiting; increased/reduced fruit size etc. at maturity stage was found to increase with increasing concentrations of mutagens in both varieties. In var. Pusa jwala it ranged between 5.33 –

10.52% and 4.11 – 9.22% in 0.01 – 0.05% MMS and DES respectively (Table 5). In var. G4 the variation frequency was recorded as 4.95 – 10.12% in 0.01 – 0.05% MMS and 4.02 – 9.12% in 0.01 – 0.05% (Table 6). The order of induction of morphological anomalies by mutagens was MMS>DES in both varieties (Pusa jwala and G4).

4.2 Meiotic studies in M_1 generation:

Cytological studies are necessary to obtain information regarding the effect of the mutagens on various genotypes. Chemical mutagens provide a very good tool for creating the alterations in the genotypes and enhance the variability in different biological parameters.

The var. Pusa jwala and G4 revealed twelve perfect bivalents (2n=24) at diakinesis (Plate I, Figs. 1&2) and metaphase-I (Plate I, Figs. 3&4) and showed normal disjunction of chromosomes (12:12) at anaphase-I (Plate I, Fig. 5). Telophase-I, anaphase-II and telophase-II were normal giving rise to normal tetrads (Plate I, Figs. 6-9). The meiosis was normal in control plants of both the varieties.

The microsporogenesis was highly disturbed in treated plants. The meiotic studies showed that the type of chromosomal aberrations were more or less similar in both the varieties but the frequencies of these chromosomal aberrations were different (Tables 9-11). The mutagenic treatments induced various chromosomal aberrations during microsporogenesis in M_1 generation. The most frequent aberrations observed were stickiness, univalents, multivalents, precocious separation, stray bivalents at metaphase-I/II and laggards, bridges, unequal separation at anaphase-I/II. The main meiotic aberrations which are commonly seen at telophase-I/II were disturbed polarity, micronuclei and cytomixis. Representative photomicrograph of cytological abnormalities are shown in the plates I-V. The Tables 9 & 10 showed that almost all types of chromosomal aberrations were dose dependent in both the varieties. The various meiotic abnormalities at different stages of meiosis were recorded as follows:

4.2.1 Metaphase I/II:

At metaphase-I/II pollen mother cells (PMCs) with univalents, multivalents, stickiness, precocious separation, stray chromosomes, were observed in MMS, and DES treated populations in varieties Pusa jwala and G4. Moreover, asynchronous movement

of chromosome were also observed in MMS and DMS treated populations of both the varieties in very low frequency (Plates I-V)

The frequency of abnormal pollen mother cells at metaphase-I/II showed dose dependent increase in treated populations and was maximum in the highest concentration of MMS and DES in both the varieties. It ranged between 2.56 to 8.49% in 0.01 – 0.05% MMS and 2.47% – 7.69% in 0.01 – 0.05% DES in var. G4 (Table 9), while in variety Pusa jwala it ranged from 3.76 to 10.22% in 0.01 – 0.05% MMS and 1.92% – 10.53% in 0.01 – 0.05% DES (Table 10).

4.2.2 Anaphase-I/II:

The abnormalities at anaphase-I/II in MMS and DES treatments were laggards, bridges and unequal separation of chromosomes in varieties Pusa jwala and G4. Moreover, PMCs showing asynchronous movement of chromosomes at anaphase-I/II and stickiness of chromosomes at anaphase-I were also observed in MMS and DES treatments in both the varieties in very low percentage (Plates II-IV).

A dose dependent increase in the frequency of pollen mother cells showing meiotic abnormalities at anaphase-I/II was observed in both the varieties The number of pollen mother cells with meiotic aberrations at anaphase-I/II increased from 2.58% to 7.24% and 1.41% to 6.50% in 0.01% – 0.05% MMS in var. G4 and Pusa jwala respectively, whereas these values in 0.01% – 0.05% DES treated populations of var. G4 and Pusa jwala were 2.49% to 7.25% and 0.48% to 5.79% respectively (Table 9 & 10).

4.2.3 Telophase-I/II:

The various chromosomal aberrations such as micronuclei, cytomixis, bridges and disturbed polarity (Plate IV-V) in both the varieties were observed at telophase-I/II. In var. G4 the number of cells with meiotic aberrations at telophase-I/II increased with increasing concentration of mutagens from 1.61 to 5.21% in 0.01% - 0.05% MMS and 1.51% to 3.16% in 0.01% to 0.05% DES (Table 9). In variety Pusa jwala the number of cells with meiotic anomalies also showed a dose dependent increase in treated populations and ranged between 1.35 to 7.31% in 0.01% - 0.05% MMS and 19.20% to 7.11% in 0.01 - 0.05% DES (Tables 10).

The results in Table 8 and 9 revealed that meiotic aberrations increased with the increase in concentration of each mutagen. MMS induced more chromosomal anomalies as compared to DES in both varieties. However, frequency of meiotic aberrations was comparatively more in var. G4 than var. Pusa jwala (Table 11).

4.3 Chiasma Frequency per cell at Metaphase-I:

The chiasma frequency at metaphase-I reduced gradually with increasing concentrations of mutagens in both varieties. In control plants chiasma frequency per cell was 18.65 which was reduced to and then reduced from 18.00 to 15.19 per cell in 0.01%-0.05% MMS, and from 18.10 to15.70 in 0.01%-0.05% DES in var. G4. In var. Pusa jwala it also decreased from 18.77 (control) to 14.20 in 0.05% MMS and 14.40 in 0.05% DES (Table 12).

4.3.1 Chiasma Frequency per bivalent at Metaphase-I:

The chiasma frequency per bivalent at metaphase-I was also reduced gradually with increasing concentration of mutagens in varieties G4 and Pusa jwala. It was 1.55 per bivalent in control which decreased from 1.51 to 1.31 per bivalent in 0.01% – 0.05% MMS and 1.50 to 1.30 per bivalent in 0.01% – 0.05% DES in var. G4 (Table 12). In var. Pusa jwala it was also decreased from 1.50 to 1.18 per bivalent in 0.01% – 0.05% MMS and from 1.53 to 1.20 in 0.01 – 0.05% as compared to 1.56 per bivalent in control (Table 12).

4.4 Studies on quantitative characters:

In M_1 generation, the effect of MMS and DES was studied on seven quantitative traits viz., days to flowering, plant height (cm), days to maturity, number of fruits per plant, fruit length (cm), fruit girth (cm) and total yield per plant (g). Data on all these polygenic traits and comparative effect of mutagens on mean values and coefficient of variation in two varieties of *Capsicum annuum* L. are summarized in Tables 13&14. A perusal of the results on various quantitative traits revealed that each trait was affected individually by the mutagens and the differential varietal response to different mutagens was also observed.

Days to flowering and days to maturity decreased in lower concentration as compared to control but increased in higher concentration in both the varieties. On the other hand,

plant height decreased with increasing concentration of both the mutagens in both the varieties. Number of fruit per plant, fruit length and fruit girth decreased in all the treatments with few exceptions where these values shifted positively over their control. Total yield per plant (g) also followed the same trend i.e., decrease over their control whereas some treatments showed stimulatory effect (Table 13&14).

The poled mean values for days to flowering and days to maturity showed slight delayed effect in both the varieties (Table 15). The pooled mean values for plant height and fruit per plant were reduced whereas pooled mean values for fruit length and fruit girth remained almost unchanged in both the varieties. Pooled mean value for total yield per plant (g) was also slightly decreased over the control in both the varieties (Table 15). The polygenic variability measured in terms of coefficient of variability (CV %) in all mutagenic treatments were found to increased as compared to control. The maximum coefficient of variability was recorded for fruits per plant followed by fruit length and fruit girth in var. Pusa jwala but in variety G4 the maximum coefficient of variability was recorded for fruit girth and fruit length followed by number of fruit per plant (Table 15).

Table 5. Effect of MMS and DES on seed germination, plant survival and pollen fertility and variation frequency in M_1 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatment (%)	Germination (%)	Inhibition (%)	Variation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	94.40	-	-	94.60	-	95.00	-
MMS							
0.01	84.33	10.66	5.33	77.15	18.44	83.10	12.52
0.02	80.53	14.70	5.95	70.39	25.60	81.65	14.05
0.03	75.62	19.90	6.88	65.82	30.42	77.40	18.52
0.04	62.83	33.45	8.11	59.52	37.08	74.35	21.73
0.05	50.95	46.02	10.52	52.11	44.91	70.19	26.11
DES							
0.01	85.15	9.80	4.11	80.45	14.95	85.32	10.18
0.02	83.70	11.34	4.93	75.30	22.51	82.26	13.42
0.03	75.12	13.34	5.54	69.45	26.58	79.46	16.35
0.04	64.48	31.70	7.90	63.10	33.30	73.70	22.42
0.05	52.44	44.45	9.22	54.77	42.10	71.85	24.36

Table 6. Effect of MMS and DES on seed germination, plant survival, pollen fertility and variation frequency in M_1 generation of *Capsicum annuum* L. var. G_4.

Treatment (%)	Germination (%)	Inhibition (%)	Variation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	92.25	-	-	95.15	-	96.00	-
MMS							
0.01	84.48	8.43	4.95	81.10	14.76	84.25	12.23
0.02	82.10	11.00	5.15	76.52	19.57	79.50	17.18
0.03	77.43	16.07	6.29	73.72	22.52	76.15	20.67
0.04	65.12	29.40	7.88	67.19	29.75	71.55	25.46
0.05	53.33	42.18	10.12	55.11	42.08	66.60	30.62
DES							
0.01	85.10	7.76	4.02	82.11	13.70	86.30	10.10
0.02	83.85	9.11	4.19	77.42	18.63	83.45	13.07
0.03	77.97	15.44	5.13	74.81	21.35	78.30	18.43
0.04	66.13	28.31	7.36	68.44	28.07	75.20	21.66
0.05	54.87	40.25	9.12	59.83	37.12	69.70	27.39

Table 7: Height of seedling raised from MMS and DES treated seeds in M_1 generation of *Capsicum annuum* L. var. Pusa jwala.

	Treatments	Length in Cm			Injury (%)
		Shoot $\bar{x} \pm SE$	Root $\bar{x} \pm SE$	Total $\bar{x} \pm SE$	
	Control	8.16 ± 0.11	2.25 ± 0.14	10.36 ± .25	-
MMS(%)	0.01	7.85 ± 0.12	2.10 ± 0.08	9.95 ± 0.20	3.80
	0.02	7.12 ± 0.17	1.95 ± 0.06	9.07 ± 0.23	12.74
	0.03	5.78 ± 0.15	1.72 ± 0.04	7.50 ± 0.19	29.16
	0.04	4.59 ± 0.11	1.49 ± 0.03	6.08 ± 0.14	43.75
	0.05	4.11± 0.13	1.19±0.06	5.30 ±0.19	49.64
DES(%)	0.01	7.95 ± 0.14	2.20 ± .22	10.15 ± 0.36	2.58
	0.02	7.35 ± 0.11	2.00 ± 0.08	9.35 ± 0.19	9.93
	0.03	6.05 ± 0.16	1.77 ± 0.04	7.82 ± 0.20	25.86
	0.04	5.15 ± 0.13	1.55 ± 0.07	6.70 ± 0.19	36.89
	0.05	4.48 ± 0.15	1.02 ± 0.09	5.50 ± 0.24	45.09

Table 8: Height of seedlings raised from MMS and DES treated seeds in M_1 generation of *Capsicum annuum* L. var. G4.

	Treatments	Length in cm			Injury (%)
		Shoot $\bar{x} \pm SE$	Root $\bar{x} \pm SE$	Total $\bar{x} \pm SE$	
	Control	8.30 ± 0.10	2.33 ± 0.14	10.63 ± .24	-
MMS (%)	0.01	8.00 ± 0.08	2.14 ±0.09	10.14 ± 0.17	3.62
	0.02	7.40± 0.10	2.02 ± 0.05	9.42 ± 0.15	10.85
	0.03	6.23 ± 0.08	1.77 ± 0.06	8.00± 0.14	25.36
	0.04	4.49 ± 0.13	1.33 ± 0.04	5.82 ± 0.17	45.90
	0.05	3.93 ± 0.11	1.09 ± 0.05	5.02 ± 0.16	52.65
DES (%)	0.01	8.05± 0.07	2.21 ± 0.08	10.26 ± 0.15	3.06
	0.02	7.51± 0.08	2.19 ± 0.09	9.70 ± 0.17	9.69
	0.03	6.34 ± 0.10	1.80 ± 0.04	8.15 ± 0.14	24.02
	0.04	4.55 ± 0.11	1.41 ± 0.05	5.96 ± 0.16	45.96
	0.05	4.10 ± 013	1.17 ± 0.06	5.30 ± 0.19	50.60

Table 9. Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_1 generation of *Capsicum annuuum* L. var. G4

Conc.	Metaphase I/II						Anaphase I/II					Telophase I/II				Total abe.
	Uni.	Multi.	Stic.	Preco.	Stray.	Total	Lag. Uneq.	Bri.		Non-syn.	Total	Micr.	Dist.	Cyto	Total	(%)
Control	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–
MMS(%)																
0.01	0.49	0.41	0.83	0.83	-	**2.56**	1.29	0.41	0.88	-	**2.58**	1.20	0.41	-	**1.61**	**6.60**
0.02	0.81	0.80	1.80	-	0.45	**3.95**	0.91	0.49	1.32	0.14	**2.86**	1.90	0.44	-	**2.34**	**9.11**
0.03	1.78	0.57	1.41	0.90	-	**5.23**	1.31	-	1.89	-	**3.20**	2.49	-	0.90	**3.39**	**11.60**
0.04	2.36	1.49	1.90	1.32	0.57	**7.68**	1.80	0.40	1.40	0.92	**4.52**	2.95	0.85	0.47	**4.27**	**16.44**
0.05	2.71	1.37	2.36	0.45	1.60	**8.49**	2.72	1.39	1.83	1.30	**7.24**	2.86	1.90	0.45	**5.21**	**23.85**
DES(%)																
0.01	0.40	0.41	0.83	0.83	-	**2.47**	1.25	0.41	0.83	-	**2.49**	1.10	0.41	-	**1.51**	**6.65**
0.02	0.96	0.90	1.80	-	0.48	**4 .14**	0.90	0.45	1.36	0.15	**2.86**	0.90	0.45	0.56	**1.91**	**8.66**
0.03	1.88	0.47	1.41	0.94	-	**4.70**	1.41	-	1.88	-	**3.29**	1.47	-	0.94	**2.41**	**11.50**
0.04	2.38	1.42	1.90	1.42	0.47	**7.59**	1.90	0.47	1.42	0.95	**4.74**	1.42	0.95	0.47	**2.84**	**15.24**
0.05	1.81	2.27	1.36	0.45	1.80	**7.69**	2.72	1.36	1.81	1.36	**7.25**	1.81	0.90	0.45	**3.16**	**20.45**

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**=precocious separation, **Stray**.=stray bivalent, **Lag.**=laggard, **Bri.**=bridge, **Uneq.**=unequal separation, **Non-syn**. = Non-synchronization of bivalents/chromosome, **Micro.**=micronuclei, **Dist.**=disturbed polarity, **Cyto.**=cytomixis, **Total abe.**= total aberration, - = not observed.

Table 10: Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_1 generation of *Capsicum annuuum* L. var. Pusa jwala.

Conc.	Metaphase I/II					Total	Anaphase I/II			Total	Telophase I/II				Total	Total Abe. (%)
	Uni.	Multi.	Stic.	Preco.	Stray.		Lag.	Bri.	Uneq.		Micr.	Dist.	Cyto.	Bri.		
Control	-	-	-	-	-		-	-	-	-	-	-	-	-	-	
MMS(%)																
0.01	-	0.94	0.94	0.47	1.41	3.76	0.94	-	0.47	1.41	-	0.41	0.94	-	1.35	6.55
0.02	0.48	1.44	0.92	0.96	0.48	4.28	0.48	-	0.96	1.42	0.48	1.44	1.92	0.48	3.96	11.06
0.03	0.93	1.86	1.86	0.46	0.93	6.04	1.39	0.93	0.47	2.79	0.93	1.39	1.86	0.93	5.11	13.96
0.04	1.36	1.96	2.27	0.90	1.36	7.85	1.81	1.36	1.81	4.98	1.27	1.81	1.36	0.92	5.36	18.09
0.05	1.86	2.32	2.79	1.39	1.86	10.22	2.32	1.86	2.32	6.50	1.79	1.91	2.32	1.86	7.31	24.06
DES(%)																
0.01	-	0.48	0.96	-	0.48	1.92	0.48	-	-	0.48	0.96	0.96	-	-	1.92	4.84
0.02	0.47	0.95	0.95	0.47	0.95	3.79	0.95	-	0.47	1.42	0.95	0.95	0.47	-	2.37	7.62
0.03	0.93	1.39	1.39	0.46	0.96	4.20	1.39	0.46	0.93	2.78	1.39	1.39	0.46	-	3.24	11.17
0.04	1.36	1.81	2.27	0.90	1.36	7.70	1.82	0.90	1.81	4.52	1.81	1.81	0.90	0.45	4.97	16.82
0.05	1.77	2.22	2.66	1.33	1.77	9.75	2.22	1.34	2.23	5.79	2.67	2.22	1.34	0.88	7.11	22.66

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**= precocious separation, **Stray.**=stray bivalent, **Lag.**=laggard, **Bri.**=bridge, **Uneq.**=unequal separation, **Micro.**=micronuclei, **Dist.**=disturbed polarity, **Cyto.**=cytomixis, - = not observed.

Table 11: Comparison of frequency of meiotic abnormalities induced by MMS and DES at different stages of meiosis in M_1 generation of *Capsicum annuum* L. var. Pusa jwala and G4

Treatments	Var. Pusa jwala			Total	Var. G4			Total
	Metaphase I/II	Anaphase I/II	Telophase I/II	Abnormalities (%)	Metaphase I/II	Anaphase I/II	Telophase I/II	Abnormalities (%)
Control	-	-	-	-	-	-	-	-
MMS (%)								
0.01	2.56	2.58	1.61	**6.60**	3.76	1.41	1.35	**6.55**
0.02	3.95	2.86	2.34	**9.11**	4.28	1.42	3.96	**11.06**
0.03	5.23	3.20	3.39	**11.60**	6.04	2.79	5.11	**13.96**
0.04	7.68	4.52	4.27	**16.44**	7.85	4.98	5.36	**18.09**
0.05	8.49	7.24	5.21	**23.85**	10.22	6.50	7.31	**24.06**
DES (%)								
0.01	2.47	2.48	1.51	**6.65**	1.92	0.48	1.92	**4.84**
0.02	4.14	2.86	1.91	**8.66**	3.79	1.42	2.37	**7.62**
0.03	4.70	3.29	2.41	**11.50**	4.20	2.78	3.24	**11.17**
0.04	7.59	4.74	2.84	**15.24**	7.70	4.52	4.97	**16.82**
0.05	7.69	7.25	3.16	**20.45**	9.75	5.79	7.11	**22.66**

Table 12: Effect of MMS and DES on chiasma frequency at Metaphase-I in M_1 generation of *Capsicum annuum* L. var. G_4 and Pusa jwala.

Treatment	Var. G4		Var. Pusa jwala	
	Chiasma/cell	Chiasma/bivalent	Chiasma/cell	Chiasma/bivalent
Control	18.65±0.22	1.55±0.06	18.77±0.19	1.56±0.07
MMS (%)				
0.01	18.00±0.19	1.51±0.04	18.10±0.15	1.50±0.06
0.02	17.65±0.20	1.47±0.06	17.30±0.10	1.44±0.04
0.03	17.11±0.16	1.43±0.07	16.80±0.11	1.40±0.08
0.04	16.31±0.13	1.38±0.04	15.10±0.13	1.25±0.06
0.05	15.19±0.19	1.31±0.06	14.20±0.16	1.18±0.04
DES (%)				
0.01	18.10±0.18	1.50±0.04	18.40±0.20	1.53±0.07
0.02	17.80±0.23	1.48±0.06	17.65±0.24	1.47±0.04
0.03	17.20±0.25	1.43±0.03	17.10±0.18	1.42±0.05
0.04	16.50±0.19	1.37±0.08	15.25±0.16	1.27±0.08
0.05	15.70±0.22	1.30±0.04	14.40±0.19	1.20±0.06

Table 13: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_1 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	48.60 ± 0.85 (8.86)	58.20 ± 0.30 (1.54)	117.40 ± 0.30 (1.42)	17.50 + 0.30 (12.88)	6.110 ± 0.22 (10.65)	2.45 ± 0.41 (8.16)	5.15 ± 0.16 (7.00)
MMS (%)							
0.01	48.15 ± 0.96 (10.42)	56.90 ± 0.41 (2.60)	117.10 ± 0.25 (1.55)	16.35 ± 0.62 (12.23)	6.25 ± 0.19 (11.90)	2.52 ± 0.13 (12.30)	5.05 ± 0.27 (9.88)
0.02	48.00 ± 0.90 (11.25)	56.10 ± 0.43 (3.22)	117.30 ± 0.51 (1.62)	19.15 ± 0.54 (15.58)	6.70 + 0.29 (13.40)	2.93 ± 0.12 (12.70)	6.30 ± 0.34 (10.34)
0.03	49.77 ± 0.75 (11.50)	55.40 ± 0.49 (4.15)	117.15 ± 0.45 (1.90)	16.70 ± 0.51 (16.70)	6.10 ± 0.43 (17.65)	2.30 ± 0.19 (15.16)	4.90 ± 0.19 (13.67)
0.04	51.10 ± 0.80 (12.50)	54.10 ± 0.73 (4.90)	118.30 ± 0.63 (2.36)	14.20 ± 0.59 (18.67)	5.95 ± 0.50 (19.30)	2.10 ± 0.40 (18.10)	4.60 ± 0.36 (14.18)
0.05	53.44 ± 0.85 (12.10)	51.30 ± 0.75 (6.20)	118.80 ± 0.52 (2.99)	12.65 ± 0.56 (21.10)	5.70 ± 0.44 (21.85)	1.90 ± 0.20 (18.95)	4.15 ± 0.42 (16.47)
DES (%)							
0.01	48.41 ± 0.76 (9.15)	57.15 ± 0.52 (3.35)	117.33 ± 0.26 (1.75)	17.00 ± 0.72 (12.45)	6.20 ± 0.22 (12.10)	2.35 ± 0.26 (10.50)	5.05 ± 0.19 (10.13)
0.02	48.32 ± 0.60 (9.35)	56.75 ± 0.54 (4.40)	117.20 ± 0.39 (1.66)	15.10 ± 0.81 (13.20)	6.30 ± 0.30 (14.20)	2.12 ± 0.19 (12.10)	4.92 ± 0.27 (11.97)
0.03	48.20 ± 0.50 (10.50)	55.90 ± 0.54 (4.50)	117.25 ± 0.52 (1.88)	19.20 ± 0.76 (13.20)	6.90 ± 0.38 (17.10)	2.89 ± 0.33 (15.15)	5.69 ± 0.34 (14.13)
0.04	50.13 ± 0.65 (11.90)	53.30 ± 0.68 (5.25)	120.10 ± 0.45 (1.62)	12.40 ± 0.67 (16.10)	6.05 ± 0.20 (17.95)	2.05 ± 0.29 (17.60)	4.56 ± 0.29 (17.12)
0.05	51.60 ± 0.75 (12.55)	51.10 ± 0.63 (6.10)	121.23 ± 0.54 (1.92)	12.10 ± 0.73 (18.60)	5.80 ± 0.45 (22.38)	1.96 ± 0.18 (19.15)	4.21 ± 0.36 (19.17)

(Figures in Parenthesis represents CV%)

Table 14: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_1 generation of *Capsicum annuum* L. var. G4.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	57.35±0.72 (6.15)	60.10±0.39 (1.85)	180.20±0.42 (1.45)	13.50±0.75 (11.90)	5.90±0.60 (11.65)	2.83±0.16 (10.17)	4.90±0.21 (9.18)
MMS(%)							
0.01	57.20±0.73 (6.25)	58.90±0.59 (2.10)	180.18±0.61 (1.54)	12.10±0.72 (13.40)	5.85±0.44 (12.15)	2.96±0.21 (12.10)	4.13±0.29 (10.92)
0.02	57.16±0.60 (8.35)	56.35±0.63 (2.70)	180.11±0.54 (1.55)	11.40±0.54 (13.90)	5.70±0.28 (14.10)	2.70±0.29 (13.19)	5.17±0.37 (12.16)
0.03	57.30±0.90 (9.55)	55.90±0.73 (2.19)	180.15±0.66 (1.90)	15.90±0.65 (16.30)	5.95±0.33 (11.90)	2.52±0.41 (16.90)	4.70±0.42 (13.97)
0.04	60.30±0.93 (10.55)	51.70±0.66 (3.60)	181.90±0.50 (2.30)	10.60±0.77 (17.70)	5.30±0.45 (15.70)	2.30±0.22 (18.60)	4.36±0.39 (15.22)
0.05	63.25±0.95 (12.60)	49.30±0.68 (4.45)	182.10±0.72 (2.45)	10.10±0.55 (16.80)	5.05±0.65 (17.65)	2.05±0.32 (21.33)	4.16±0.29 (18.33)
DES(%)							
0.01	57.20±0.50 (7.30)	58.25±0.30 (3.82)	180.18±0.58 (1.45)	12.80±0.62 (12.30)	5.75±0.19 (11.90)	2.79±0.19 (11.20)	4.80±0.19 (9.53)
0.02	57.15±0.46 (8.48)	57.55±0.51 (4.10)	180.10±0.43 (1.48)	14.60±0.84 (15.10)	5.40±0.25 (14.15)	2.60±0.23 (13.10)	4.69±0.27 (4.15)
0.03	57.25±0.65 (10.15)	55.70±0.63 (4.50)	180.17±0.55 (1.59)	11.10±0.76 (17.50)	6.10±0.43 (17.90)	3.05±0.16 (17.47)	5.05±0.34 (13.67)
0.04	61.81±0.72 (11.15)	54.10±0.43 (5.25)	182.90±0.61 (1.45)	10.30±0.80 (20.70)	5.10±0.36 (20.28)	2.45±0.39 (21.12)	4.51±0.26 (16.13)
0.05	63.20±0.75 (11.78)	50.30±0.42 (6.10)	185.40±0.77 (2.35)	9.90±0.72 (22.20)	4.90±0.22 (23.15)	2.11±0.27 (23.44)	4.20± 0.35 (18.19)

(Figures in Parenthesis represents CV%)

Table 15: Comparative effect of mutagens on mean and coefficient of variation in M_1 generation of *Capsicum annuum* L. var. Pusa jwala and G4.

Characters	Var. Pusa jwala			Var. G4		
	Control	MMS	DES	Control	MMS	DES
	$\bar{x} \pm S.E$ (CV%)	$\bar{x} \pm S.E$ (CV %)	$\bar{x} \pm S.E$ (CV%)	$\bar{x} \pm S.E$ (CV %)	$\bar{x} \pm S.E$ (CV %)	$\bar{x} \pm S.E$ (CV%)
Days to Flowering	48.60±0.85(8.86)	50.09±0.85(11.55)	49.33±0.65(10.68)	57.30±0.72(6.15)	58.94±0.82(9.46)	59.32±0.60(9.57)
Days to maturity	117.40±0.30(1.42)	117.33±0.49(2.08)	118.63±0.43(1.76)	180.20±0.42(1.45)	180.88±0.60(2.55)	181.75±0.59(1.74)
Plant height	58.20±0.30(1.54)	55.76±0.5(4.21)	55.03±0.59(4.71)	60.10±.39(1.85)	55.44±0.65(3.01)	55.18±0.46(4.75)
Fruit per plant	17.60±0.30(12.88)	15.81±0.56()16.85	15.16±0.73(14.79)	13.50±0.75(11.90)	12.02±0.64(16.23)	11.74±0.74(17.26)
Fruit length	6.40±0.22(10.65)	6.14±0.38(16.82)	6.25±0.31(16.75)	5.90±0.60(11.65)	5.57±0.43(14.30)	5.45±0.45(17.47)
Fruit girth	2.45±0.41(8.16)	2.35±0.21(15.44)	2.27±0.25(14.90)	2.83±0.16(10.17)	2.50±0.29(16.34)	2.60±0.25(17.56)
Total yield per plant	5.15±0.16(7.00)	5.00±0.32(12.90)	4.88±0.29(14.42)	4.90±0.21(9.18)	4.50±0.35(14.12)	4.65±0.28(13.74)

4.5 STUDIES IN M_2 GENERATION:

4.5.1 Seed germination:

The seed germination in untreated population (control) of var. Pusa jwala was 93.78% which decreased to 54.95% in 0.05% MMS and 55.54% in 0.05% DES respectively (Table 16). The seed germination in var. G4 was 92.45% (control). The percentage of seed germination was decreased to 56.39% in 0.05% MMS and 59.67% in 0.05% DES (Table 17). Although, there was decreasing trend in seed germination with the increasing concentrations of both the mutagens but considerable recovery was noted in M_2 generation resulting the higher percentage of seed germination in M_2 than M_1 generation. The maximum inhibition in seed germination was 41.40% in 0.05% MMS followed by 40.78% in 0.05% DES in var. Pusa jwala (Table 16), whereas, in var. G4 it was 35.45% in 0.05% MMS and 35.45% in 0.05% DES (Table 17). The germination was comparatively more affected in var. Pusa jwala in M_2 generation also.

4.5.2 Plant Survival:

Although, the survival of plants decreased with the increasing concentration of both the mutagens in both varieties viz. Pusa jwala and G4, but it was higher as compared to M_1 generation (Table 15). The maximum survival percentage was observed at the lowest concentration and decreased linearly in higher concentration of both mutagens from 95.00% (control) to 61.40% in 0.05% MMS and 63.25% in 0.05% DES in var. Pusa jwala (Table 16). In var. G4 survival percentage was reduced linearly from 93.25% (control) to 67.55% in 0.05% MMS and 68.95% in 0.05% DES (Table17).The highest lethality (35.36% and 27.56%) were recorded at 0.05% MMS in the var. Pusa jwala and G4 respectively (Table 16&17). Var. Pusa jwala was found to be more sensitive than var. G4 as the survival in Pusa jwala was more adversely affected by mutagens and that MMS was more affective in inducing lethality.

4.5.3 Pollen Fertility:

The pollen fertility was affected by mutagens in M_2 generation also. It reduced with the increasing concentrations of both the mutagens. The pollen fertility in controls was 94.25%% and 94.56% in Pusa jwala and G4 respectively. The range of decrease in fertility was from 83.68 – 67.17% in 0.01 – 0.05% MMS and 85.43 – 70.94 % in 0.01 – 0.05% DES in variety Pusa jwala (Table 16), while in var. G4 it ranged between 83.68 – 67.17% and 86.30 – 70.11% in 0.01-0.05% MMS and DES respectively (Table 17). The MMS was highly effective on pollen fertility as compared to DES in both the varieties. Consequently the relative reduction in pollen fertility was found maximum i.e., 27.38% in 0.05% MMS followed by 23.31% in 0.05% DES in var. Pusa jwala (Table 16). Similarly in var. G4 the relative reduction in pollen fertility was maximum i.e.,28.96 in 0.05% MMS followed by 25.85% in 0.05% DES (Table 17). Both the varieties responded differently to mutagens. However, var. Pusa jwala was more affected.

4.5.4 Frequency of Mutations at Older Stage:

The mutations, at maturity stage of the plant, were found to increase in increasing concentrations of both the mutagens. In control populations of both varieties the variations were absent, while increased from 2.38 – 3.91% and 1.48 – 2.73% in 0.01– 0.05% MMS and DES respectively in var. Pusa jwala (Table 16). In var. G4 the mutation frequency increased from 1.83 – 2.92% and 0.88 – 2.97% in 0.01– 0.05% MMS and DES respectively in var. G4 (Table 17). The variations, which were noted at older plant stage in M_1 generation such as Tall/dwarf, stunted/improved growth; improved/poor branching and fruiting; increased/reduced fruit size etc. were inherited in M_2 also, but the frequency was lesser than M_1 generation.

4.6 MEIOTIC STUDIES IN M_2 GENERATION:

Cytological analysis in M_2 generation was carried out in control as well as plants raised from treated seeds obtained from M_1 generation. The types of chromosomal aberrations were more or less similar, but the frequency of various aberrations was

different in both the varieties (Table 18&19). Various meiotic abnormalities at different stages of meiosis were recorded as follows.

4.6.1 Metaphase-I/II:

The number of cells showing meiotic aberrations at metaphase-I/II showed a dose dependent increase in treated populations and it increased from 1.86 to 7.55% in 0.01 – 0.05% MMS and 1.82 to 7.06% in 0.01 – 0.05% DES in var. G4 (Table 18). In var. Pusa jwala, the number of abnormal cells increased from 3.39 to 9.56% in 0.01 – 0.05% MMS and 1.28 to 8.85% in 0.01 – 0.051% DES (Table 19).

4.6.2 Anaphase-I/II:

The frequency of pollen mother cells showing meiotic abnormalities at anaphase-I/II ranged from 2.10 to 6.53% in 0.01 – 0.05% MMS and 2.07 to 6.75% in 0.01 – 0.05% DES in var. G4 (Table 18). In var. Pusa jwala the percentage of abnormal cells increased from 1.13 to 6.02% in 0.01 – 0.05% MMS and 0.44 to 5.28% in 0.01 – 0.05% DES (Table 19). At anaphase I/II, the chromosomal aberrations were also increased with the increase in the concentration of mutagens in both the varieties.

4.6.3 Telophase-I/II:

The frequency of pollen mother cells showing meiotic abnormalities at telophase-I/II showed dose dependent increase in treated populations from 1.44 to 4.80% in 0.01 - 0.05% MMS and 1.43 to 2.68% in 0.01 - 0.05% DES in var. G4 (Table 18). In var. Pusa jwala the percentage of PMCs showing meiotic abnormalities was increased from 1.03 to 5.27% in 0.01 – 0.05% MMS and 1.02 to 4.80% in 0.01 – 0.05% DES (Table 19). No abnormal PMCs were observed at any stages of meiosis in control plants of both the varieties.

Perusal of the results in Table 20 revealed that meiotic aberrations increased with the increase in the concentration of each mutagen in both the varieties. The overall frequency of meiotic aberrations at various stages of meiosis indicated that aberrations were more

common at metaphase followed by aberration at anaphase and telophase stages. The frequency of meiotic aberrations was comparatively more in var.G4 than var. Pusa jwala. Moreover, the frequency of meiotic aberrations recorded in M_2 was less than M_1.

4.7 Chiasma frequency per cell and per bivalent at Metaphase-I:

PMCs from treated as well as untreated (control) plants were analysed and chiasmata per cell and per bivalent was calculated (Table 21). The chiasma frequency at metaphase-I was found to be reduced with increasing concentration of both mutagens in var. Pusa jwala. In control plants the chiasma frequency was 18.78 per cell and 1.56 per bivalent. It decreased from 18.22 to 15.33 per cell and 1.52 to 1.27 per bivalent in 0.01% – 0.05% MMS treatments while in 0.01% –0.05% DES treatments it reduced from 18.39 to 15.96 per cell and 1.53 to 1.33 per bivalent (Table 21). In var. G4 chiasma frequency in control plants was recorded as 18.81 per cell and 1.51 and per bivalent. The chiasma frequency reduced from 18.19 to 14.58 per cell and 1.52 to 1.21 per bivalent in 0.01 – 0.05% MMS treatment while in 0.01% – 0.05% DES treatments it reduced from 18.53 to 14.62 per cell and 1.54 to 1.22 per bivalent (Table 21).

4.8 Quantitative characters:

Mutagenic effects of MMS and DES were studied on the seven quantitative characters in M_2 generation. The data of each character was statistically analyzed to assess the extent of induced variation by different mutagenic treatments.

4.8.1 Days to flowering and days to maturity:

The data on days to flowering and days to maturity in M_2 generation are presented in the Tables 22 & 23. In var. Pusa jwala the mean value for days to flowering was slightly decreased in lower concentrations as compared to control but significantly increased in higher concentration (0.04% – 0.05%) of both the mutagens (Table 22). Similarly in var. G4 days to flowering was significantly increased at higher concentration of MMS and DES (Tables 23). The mean value for days to maturity also slightly decreased in lower and intermediate concentration of MMS and DES as compare to control whereas, the

higher concentration significantly increased the days to maturity in var. Pusa jwala. The days to maturity slightly decreased with the lower concentration of MMS and DES. In var. G4, days to maturity was significantly delayed with higher concentrations (0.05% MMS and 0.04% and 0.05% concentrations of DES.

4.8.2 Height of Mature Plants:

The height of the plant were measured when the growth of the plants were stopped and the fruits were mature. The observation showed that the average plant height decreased with increased concentration of the mutagens. The average height in control was 58.48 cm which reduced to 57.93– 52.76 cm in 0.01-0.05% MMS and 58.12 to 53.29 cm in 0.01- 0.05% DES in var. Pusa jwala (Table 22). In var. G4 the average plant height was 60.83cm which was decreased to 58.97 – 50.49 cm in 0.01– 0.05% MMS treatment and from 58.34 to 51.76 cm in 0.01-0.05% DES treatment (Table 23). The highest values of CV (%) were observed in the highest concentrations of both mutagens in var. Pusa jwala and G4 (Table 22& 23).

4.8.3 Number of fruit/plant, fruit size and total yield per plant:

In var. Pusa jwala the mean values of number of fruit per plant and average fruit length was increased from 17.61 and 6.23cm in control to 17.93 and 7.10cm respectively in 0.02% MMS, while in higher concentration the decreasing trend was recorded from 16.48–15.30 and 6.14 – 5.89cm in 0.03-0.05% MMS treatment respectively (Table 22). The yield per plant was also increased significantly at 5% level from control i.e., 5.26g to 5.82g in 0.02% MMS (Table 22). In DES treatments the average number of fruit per plant, fruit length and total yield per plant were decreased in 0.01%, 0.02%, 0.04 and 0.05% concentrations over their respective controls. However, 0.03% DES increased the number of fruit per plant, fruit length from 17.61and 6.23 in their respective control to 19.83 and 6.55. The average yield per plant was also increased from 5.26g (control) to 5.93g (0.03% DES) at 5 % level in var. Pusa jwala (Table 22).

In var. G4, there was an increase in the average number of fruit per plant, average fruit length and total yield per plant from 14.10, 6.43cm and 5.11g in their respective control to 14.93, 6.97cm and 5.53g in 0.02% MMS treatment, while in higher concentration a decreasing trend was recorded from 12.15 – 10.34, 5.94-5.15cm, 2.63 – 2.31cm and 4.89 – 4.39g in 0.03 – 0.05% MMS (Table 23). In 0.03% DES treatments the number of fruit per plant, fruit length and total yield per plant were increased over the control while in higher concentration (0.04 – 0.05%) of DES these values decreased as compare to the control (Table 23). The lower concentration of MMS and DES i.e., 0.01% showed slightly decreasing effect on all these parameters in both the varieties (Table 22 & 23).

The maximum values of CVs were recorded in higher concentrations of both the mutagens for the above mentioned parameters in var. Pusa jwala and G4. The higher CVs% in concentrations of mutagens showed the increased variations in the population which may lead to increased diversity and better chance for screening of better mutants.

4.9. Mutagenic Effectiveness and Efficiency:

The effectiveness decreased with an increase in concentrations of MMS in var. Pusa jwala. There was a sharp decrease from 19.83 to 8.77 in 0.01 – 0.03% MMS followed by minor decrease in sill higher concentration (Table 24). Similar trend occurred in DES treatments and it decreased sharply from 12.33 – 4.55 in 0.01 – 0.03% concentration and slightly decrease further in higher concentration (Table 24). In var. G4 also the same trend was observed and it decreased from 15.25 – 4.87 in 0.01 – 0.05% MMS, and in DES treatment also, effectiveness increased in lower concentration and then decreased in higher concentration. The most effective concentrations were: 0.01% MMS and 0.01% DES in var. Pusa jwala and 0.01% MMS and 0.02% DES in var. G4 (Table 24 & 25).

The mutagenic efficiency with regard to inhibition was more in lower concentration of MMS (0.01%) and DES (0.01%) and thereafter it decreased in higher concentration in var. Pusa jwala (Table 24). In var. G4 the efficiency was found maximum in 0.01% MMS and 0.02% DES and decreased further in higher concentration (Table 25). In var. Pusa

jwala the mutagenic efficiency based on pollen sterility (Mp/S) was highest in lower concentration of MMS (0.01%) and DES (0.02%) and thereafter it decreased in higher concentration (Table 24). In var. G4 the mutagenic efficiency (Mp/S) was recorded maximum in 0.02% MMS and 0.02% DES and decreased further in higher concentration (Table 25). The most efficient concentrations with regard to pollen sterility were: 0.01% MMS and 0.02% DES in var. Pusa jwala (Table 24), and 0.02% MMS and 0.02% DES in var. G4 (Table 25).

4.10 SELECTED MUTANTS IN M_2 GENERATION:

To understand the response of two varieties of *Capsicum annuum* L. to different mutagenic treatments, the control and treated population as well as variants selected in M_1 generation were screened carefully to identify different type of viable morphological mutants at different stages of growth in M_2 generation. A comparative account of quantitative characters of all the selected mutants from both the varieties with their control plants has been presented in Table 26 & 27.

4.10.1 SELECTED MUTANT IN VAR. PUSA JWALA

4.10.1.1 Selection from MMS treatments:

Five mutants were selected from different MMS treatments in M_2 generation (Table 26).

Selection-I. It showed increased height, increase in fruit size and improved yield.

Selection-II. Plant tall and showed increased number of fruits.

Selection-III. Mutants showed early maturing fruits.

Selection-IV. Dwarf mutant.

Selection-V. Sterile mutant which did not set any fruit.

4.10.1.2 Selection from DES treatments::

Four mutants were selected from var. Pusa jwala treated with different concentrations of DES (Table 26).

Selection-I. Tall plant with large size of fruits and improved yield.

Selection-II. Semi dwarf mutant with long, thick and increased number of fruit.

Selection-III. Mutant which showed high yield and late maturity of fruits.

Selection-IV. Dwarf plant with long thin fruits.

4.10.2 SELECTED MUTANT IN VAR. G4.

4.10.2.1 Selection from MMS treatment:

Four different types of mutants were isolated from population treated with different concentration of MMS (Table 27).

Selection-I. Tall plant with short fruit size.

Selection-II. Mutant with long, slender and thick fruits.

Selection-III. Tall mutant, early maturing fruits and improved yield.

Selection-IV. Dwarf plant with seedless fruits.

4.10.2.2 From DES treatment:

Three mutants were isolated in M_2 generation from 0.02%, and 0.03% concentration of DES (Table 27).

Selection-I. Mutant with large number of fruits showing increased yield.

Selection-II. Semi-dwarf mutant with long fruits and increased yield.

Selection-III. Late maturing mutant with thick fruits and increased yield.

Table 16. Effect of MMS and DES on seed germination, mutation frequency, plant survival and pollen fertility in M_2 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatment (%)	Germination (%)	Inhibition (%)	Mutation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	93.78	-	-	95.00	-	94.25	-
MMS							
0.01	87.23	6.98	2.38	81.20	14.73	84.90	11.83
0.02	82.95	11.55	2.55	77.80	18.10	80.13	13.36
0.03	78.32	16.49	3.16	74.60	21.47	77.68	17.87
0.04	69.63	25.76	3.63	70.10	26.21	72.34	21.11
0.05	54.95	41.40	3.91	61.40	35.36	68.44	27.38
DES							
0.01	88.69	5.43	1.48	83.50	12.10	85.43	09.35
0.02	86.10	8.19	2.09	81.20	14.52	83.97	10.90
0.03	76.92	17.98	2.26	75.30	21.15	79.46	16.35
0.04	67.88	27.61	2.63	72.50	23.90	75.81	19.56
0.05	55.54	40.78	2.73	63.25	33.42	70.94	23.31

Table 17. Effect of MMS and DES on seed germination, mutation frequency, plant survival and pollen fertility in M_2 generation of *Capsicum annuum* L. var. G_4.

Treatment (%)	Germination (%)	Inhibition (%)	Mutation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	92.45	-		93.25	-	94.56	-
MMS							
0.01	88.13	4.68	1.83	83.90	10.02	83.68	11.50
0.02	86.53	6.41	2.23	78.30	16.03	81.75	13.54
0.03	80.93	12.47	2.47	76.40	18.06	78.11	17.39
0.04	69.12	25.24	2.68	69.90	25.05	73.39	22.38
0.05	56.39	39.00	2.92	67.55	27.56	67.17	28.96
DES							
0.01	88.75	4.00	0.88	84.50	9.39	86.30	08.76
0.02	86.85	6.05	1.82	79.30	14.95	84.58	10.55
0.03	81.91	11.40	1.83	76.85	17.58	80.44	14.93
0.04	71.63	22.53	2.53	72.10	22.69	74.92	20.76
0.05	59.67	35.45	2.97	68.95	26.05	70.11	25.85

Table 18. Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_2 generation of *Capsicum annuuum* L. var. G4

Conc.	Metaphase I/II					Total	Anaphase I/II				Total	Telophase I/II			Total	Total abe. (%)
	Uni.	Multi.	Stic.	Preco.	Stray.		Lag.	Bri.	Uneq.	Non-syn.		Micr.	Dist.	Cyto		
Control	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–
MMS(%)																
0.01	0.30	0.33	0.63	0.60	-	1.86	1.13	0.29	0.68	-	2.10	1.11	0.33	-	1.44	5.40
0.02	0.69	0.71	1.59	-	0.34	3.33	0.71	0.34	1.22	0.11	2.38	1.73	0.24	0.12	2.09	7.80
0.03	1.58	0.44	1.21	0.72	0.12	4.07	1.28	-	1.59	0.15	3.02	2.29	0.11	0.68	3.08	10.17
0.04	2.16	1.33	1.63	1.22	0.27	6.61	1.63	0.34	1.26	0.76	3.99	2.75	0.65	0.37	3.77	14.37
0.05	2.53	1.19	2.18	0.39	1.26	7.55	2.51	1.26	1.63	1.13	6.53	2.76	1.79	0.25	4.80	18.88
DES(%)																
0.01	0.30	0.31	0.63	0.58	-	1.82	1.12	0.31	0.53	0.11	2.07	1.05	0.23	0.15	1.43	5.32
0.02	0.77	0.63	1.38	0.14	0.28	3.20	0.71	0.45	1.16	0.10	2.42	0.70	0.35	0.46	1.61	7.23
0.03	1.68	0.47	1.12	0.53	0.21	4.01	1.31	-	1.68	-	2.99	1.17	0.23	0.54	1.94	8.94
0.04	2.21	1.22	1.72	1.22	0.37	6.74	1.73	0.37	1.18	0.85	4.13	1.32	0.80	0.37	2.49	13.36
0.05	1.61	2.07	1.16	0.45	1.77	7.06	2.52	1.26	1.71	1.26	6.75	1.59	0.71	0.38	2.68	16.49

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**=precocious separation, **Stray**.=stray bivalent, **Lag.**=laggard, **Bri**.=bridge, **Uneq.**=unequal separation, **Non-syn**. = Non-synchronization of bivalents/chromosome, **Micro.**=micronuclei, **Dist**.=disturbed polarity, **Cyto.**=cytomixis, **Total abe.**= total aberration, - = not observed.

Table 19: Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_2 generation *Capsicum annuuum* L. var. Pusa jwala.

Conc.	Metaphase I/II					Total	Anaphase I/II			Total	Telophase I/II			Total	Total Abe. (%)
	Uni.	Multi.	Stic.	Preco.	Stray.		Lag.	Bri.	Uneq.		Micr.	Dist.	Cyto.		
Control	–	–	–	–	–		–	–	–	–	–	–	–	–	–
MMS(%)															
0.01	0.23	0.74	0.74	0.37	1.31	**3.39**	0.76	–	0.37	**1.13**	0.12	0.21	0.70	1.03	**5.55**
0.02	0.58	1.44	0.72	0.86	0.38	**3.98**	0.34	0.11	0.79	**1.24**	0.28	1.24	1.52	3.04	**8.26**
0.03	0.73	1.76	1.77	0.36	0.79	**5.41**	1.29	0.73	0.47	**2.49**	0.63	1.19	1.41	3.23	**11.13**
0.04	1.24	1.81	2.13	0.75	1.26	**7.19**	1.72	1.23	1.67	**4.62**	1.12	1.65	1.18	3.95	**15.76**
0.05	1.80	2.22	2.59	1.29	1.66	**9.56**	2.19	1.61	2.22	**6.02**	1.64	1.51	2.12	5.27	**20.85**
DES(%)															
0.01	0.13	0.21	0.66	0.10	0.18	**1.28**	0.11	0.14	0.19	**0.44**	0.56	0.36	0.10	1.02	**2.74**
0.02	0.37	0.75	0.85	0.37	0.75	**3.09**	0.69	0.13	0.27	**1.09**	0.65	0.75	0.27	1.67	**5.85**
0.03	0.73	1.09	1.24	0.26	0.76	**4.08**	1.19	0.16	0.73	**2.08**	1.19	1.29	0.16	2.64	**8.80**
0.04	1.16	1.61	2.11	0.77	1.19	**6.84**	1.62	0.75	1.59	**3.96**	1.71	1.51	0.69	3.91	**14.71**
0.05	1.57	2.02	2.56	1.13	1.57	**8.85**	2.10	1.14	2.04	**5.28**	2.10	1.67	1.03	4.80	**18.93**

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**= precocious separation, **Stray.**=stray bivalent, **Bri.**=bridge, **Lag.**=laggard, **Uneq.**=unequal separation, **Micro.**=micronuclei, **Dist.**=disturbed polarity, **Cyto.**=cytomixis, Total Abe.= Total Aberrations, - = not observed.

Table 20: Comparison of frequency of meiotic abnormalities induced by MMS and DES at different stages of meiosis in M_2 generation of *Capsicum annuum* L. var. G4 and Pusa jwala.

Treatments	Var. Pusa jwala			Total Abnormalities (%)	Var. G4			Total Abnormalities (%)
	Metaphase I/II	Anaphase I/II	Telophase I/II		Metaphase I/II	Anaphase I/II	Telophase I/II	
Control	-	-	-	-	-	-	-	-
MMS (%)								
0.01	1.86	2.10	1.44	**5.40**	3.39	1.13	1.03	**5.55**
0.02	3.33	2.38	2.09	**7.80**	3.98	1.24	3.04	**8.26**
0.03	4.07	3.02	3.08	**10.17**	5.41	2.49	3.23	**11.13**
0.04	6.61	3.99	3.77	**14.37**	7.19	4.62	3.95	**15.76**
0.05	7.55	6.53	4.80	**18.88**	9.56	6.02	5.27	**20.85**
DES (%)								
0.01	1.82	2.07	1.43	**5.32**	1.28	0.44	1.02	**2.74**
0.02	3.20	2.42	1.61	**8.23**	3.09	1.09	1.67	**5.85**
0.03	4.01	2.99	1.94	**7.94**	4.08	2.08	2.64	**8.80**
0.04	6.74	4.13	2.49	**13.36**	6.84	3.96	3.91	**14.71**
0.05	7.06	6.75	2.68	**16.49**	8.85	5.28	4.80	**18.93**

Table 21- Effect of MMS and DES on chiasma frequency at Metaphase-I in M_2 generation of *Capsicum annuum* L. var. Pusa jwala and G_4.

Treatment	Var. Pusa jwala		Var. G_4	
	Chiasma/cell	Chiasma/bivalent	Chiasma/cell	Chiasma/bivalent
Control	18.78±0.14	1.56±0.07	18.81±0.17	1.57±0.06
MMS (%)				
0.01	18.22±0.13	1.52±0.05	18.19±0.14	1.52±0.07
0.02	17.80±0.11	1.49±0.06	17.45±0.11	1.46±0.05
0.03	17.43±0.14	1.45±0.08	16.97±0.10	1.42±0.09
0.04	16.56±0.17	1.38±0.05	15.39±0.12	1.28±0.06
0.05	15.33±0.12	1.27±0.07	14.58±0.14	1.21±0.08
DES (%)				
0.01	18.39±0.16	1.53±0.04	18.53±0.16	1.54±0.08
0.02	17.94±0.19	1.49±0.07	17.81±0.23	1.48±0.06
0.03	17.43±0.21	1.45±0.05	17.26±0.17	1.44±0.05
0.04	16.81±0.15	1.40±0.08	15.43±0.15	1.28±0.09
0.05	15.96±0.21	1.33±0.06	14.62±0.18	1.22±0.08

Table 22: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_2 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	48.78±0.76(8.77)	58.48±0.33(1.55)	116.62±0.30(1.33)	17.61±0.27(12.76)	6.23±0.20(10.70)	2.35±0.36(8.11)	5.26±0.16(6.83)
MMS(%)							
0.01	48.86±0.85(10.13)	57.93±0.45(2.54)	116.59±0.32(1.46)	16.95±0.49(12.00)	6.19±0.17(11.81)	2.52±0.10(12.19)	5.20±0.22(8.30)
0.02	49.20±0.79(10.78)	57.13±0.39(3.00)	116.67±0.55(1.57)	17.93±0.55(14.81)	7.10±0.25(12.77)	2.30±0.16(12.57)	5.82*±0.30(10.14)
0.03	50.15±0.67(11.05)	56.29*±0.42(4.34)	119.10±0.40(1.81)	16.48*±0.48(16.19)	6.14±0.38(17.81)	2.01±0.23(14.87)	5.10±0.19(11.49)
0.04	50.79*±0.74(11.87)	55.31**±0.64(4.77)	122.60*±0.61(2.45)	16.13*±0.57(18.34)	6.00±0.43(18.30)	1.81±0.39(16.13)	4.93±0.29(13.68)
0.05	52.17**±0.80(12.70)	52.76**±0.70(6.87)	124.80**±0.56(3.23)	15.30**±0.56(21.59)	5.89**±0.48(21.41)	1.76±0.26(18.45)	4.21**±0.38(14.51)
LSD at 5%	1.42*	2.13*	3.10*	1.12*	0.94*	NS	0.51*
LSD at 1%	2.49**	3.13**	4.05**	1.65**	1.33**	NS	0.89**
DES(%)							
0.01	48.83±0.61(9.10)	58.12±0.48(3.11)	116.71±0.23(1.71)	17.05±0.51(12.47)	6.22±0.22(12.14)	2.30±0.21(9.80)	5.11±0.17(9.33)
0.02	48.80±0.56(9.39)	57.15±0.51(4.17)	117.10±0.31(1.60)	16.48±0.71(13.11)	6.10±0.33(14.11)	2.13±0.23(11.23)	5.06±0.23(11.17)
0.03	49.30±0.59(10.07)	56.70±0.61(4.29)	117.87±0.45(1.90)	19.83±0.64(12.93)	6.55±0.38(16.84)	2.70±0.30(13.76)	5.93*±0.30(15.20)
0.04	49.89*±0.68(12.34)	55.47*±0.59(5.20)	120.13*±0.48(1.55)	15.30*±0.67(16.33)	6.13±0.26(18.94)	2.15±0.26(16.45)	4.76±0.26(16.32)
0.05	50.33**±0.56(12.97)	53.29**±0.63(6.33)	123.42**±0.50(1.88)	13.10**±0.70(18.76)	5.23*±0.39(22.78)	1.99±0.19(15.77)	4.34**±0.30(18.54)
LSD at 5%	1.22*	2.99*	3.17*	1.98*	0.88*	NS	0.57*
LSD at 1%	1.51**	4.11**	4.11**	2.70**	0.99**	NS	0.90**

Figures in Parenthesis represents CV%, S.E.= Standard Error, LSD=Least Significant difference.

Table 23: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_2 generation of *Capsicum annuum* L. Var. G4.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	57.30±0.61 (6.09)	60.83±0.41 (1.79)	180.10±0.37 (1.41)	14.10±0.70 (11.44)	6.43±0.61 (11.29)	2.85±0.17 (10.31)	5.11±0.21 (9.23)
MMS (%)							
0.01	57.50±0.64 (6.17	58.97±0.44 (2.12)	180.60±0.53 (1.50)	13.67±0.63 (13.40)	5.93±0.45 (11.81)	2.79±0.21 (12.02)	5.0±0.27 (10.73)
0.02	57.80±0.59 (8.26)	56.81±0.59 (2.33)	179.33±0.50 (1.31)	14.93±0.56 (14.12)	6.97±0.39 (14.06)	2.89±0.25 (13.23)	5.53*±0.29 (12.09)
0.03	58.10±0.83 (9.13)	55.97*±0.74 (2.05)	180.05±0.68 (1.74)	12.15*±0.61 (16.11)	5.95±0.30 (12.13)	2.63±0.39 (16.11)	4.89±0.38 (13.61)
0.04	59.90*±0.86 (9.96)	52.89*±0.60 (3.73)	180.93±0.52 (2.33)	11.19**±0.71 (18.15)	5.64±0.41 (15.19)	2.44±0.20 (19.21)	4.57±0.33 (15.29)
0.05	63.25**±0.95 (12.34)	50.49**±0.70 (4.68)	181.97*±0.69 (2.54)	10.34**±0.50 (19.73)	5.15**±0.59 (17.68)	2.31±0.30 (21.33)	4.39**±0.30 (17.35)
LSD at 5%	1.27*	4.77*	1.13*	1.33*	0.98*	NS	0.41*
LSD at 1%	2.66**	7.88**	1.94**	2.69**	1.22**	NS	0.63**
DES (%)							
0.01	57.33±0.44 (7.13)	58.34±0.31 (3.82)	180.77±0.54 (1.47)	13.80±0.60 (11.57)	6.10±0.17 (11.50)	2.84±0.17 (11.13)	5.10±0.15 (9.50)
0.02	57.45±0.40 (8.00)	57.68±0.48 (4.13)	178.69±0.41 (1.40)	12.13±0.73 (14.81)	5.81±0.23 (14.11)	2.78±0.20 (12.59)	4.92±0.21 (11.15)
0.03	57.93±0.62 (9.54)	56.31±0.57 (3.86)	180.19±0.50 (1.54)	15.85±070 (17.11)	6.87±0.44 (17.31)	2.93±0.19 (16.18)	5.64*±0.34 (13.51)
0.04	59.12*±0.68 (1.43)	55.39*±0.44 (5.57)	182*.53±0.59 (1.43)	11.87*±0.75 (19.70)	5.43*±0.38 (21.11)	2.56±0.36 (19.12)	4.61±0.23 (16.19)
0.05	63.20**±0.68 (11.70)	51.67**±0.70 (2.29)	183.19**±0.70 (2.29)	10.23**±0.68 (21.51)	4.96**±0.20 (23.44)	2.23±0.20 (22.26)	4.26**±0.30 (18.54)
LSD at 5%	1.77*	5.11*	2.34*	2.13*	0.94*	NS	0.51*
LSD at 1%	3.19**	6.51**	2.96**	3.66**	1.39**	NS	0.73**

Figures in Parenthesis represents CV%, S.E. = Standard Error, LSD=Least Significant difference.

Table 24. Effectiveness and Efficiency of MMS and DES treatments in *Capsicum annuum* L. var. Pusa jwala.

Treatment (%)	% Mutant Plant Progenies in M2 (Mp)	% Inhibition in Germination (I)	% Pollen Sterility (S)	Mutagenic Effectiveness (Mp/CT)	Mutagenic Efficiency	
					Mp/I	Mp/S
Control	-	-	-	-	-	-
MMS						
0.01	2.38	6.98	11.83	19.83	0.34	0.20
0.02	2.55	11.55	13.36	10.62	0.22	0.19
0.03	3.16	16.49	17.87	8.77	0.19	0.18
0.04	3.63	25.76	21.11	6.47	0.14	0.17
0.05	3.91	41.40	27.38	5.15	0.09	0.14
DES						
0.01	1.48	5.43	09.35	12.33	0.27	0.15
0.02	2.09	8.19	10.90	8.70	0.25	0.19
0.03	2.26	17.98	16.35	6.28	0.12	0.14
0.04	2.63	27.61	19.56	5.39	0.09	0.13
0.05	2.73	40.78	23.31	4.55	0.06	0.11

Table 25. Effectiveness and Efficiency of MMS and DES treatments in *Capsicum annuum* L. var. G4.

Treatment (%)	% Mutant Plant Progenies in M2 (Mp)	% Inhibition in Germination (I)	% Pollen Sterility (S)	Mutagenic Effectiveness Mp/CT	Mutagenic Efficiency	
					Mp/I	Mp/S
Control	-	-	-	-	-	-
MMS						
0.01	1.83	4.68	11.50	15.25	0.39	0.15
0.02	2.23	6.41	13.54	8.67	0.32	0.16
0.03	2.47	12.47	17.39	6.86	0.19	0.14
0.04	2.68	25.24	22.38	5.58	0.10	0.12
0.05	2.92	39.00	28.96	4.87	0.07	0.10
DES						
0.01	0.88	4.00	08.76	7.33	0.22	0.10
0.02	1.82	6.05	10.55	7.58	0.30	0.17
0.03	1.83	11.40	14.93	5.08	0.16	0.13
0.04	2.53	22.53	20.76	5.27	0.11	0.13
0.05	2.97	35.45	22.85	4.51	0.08	0.12

Table 26: Selected mutants in var. Pusa jwala (M_2 Generation).

Code	Treatments	Days to-flowering	Plant-height	Days to maturity	Fruit/plant	Fruit-length (cm)	Fruit girth (cm)	Total yield/plant (g)	Important Characters
-	Control	48.78	58.48	116.62	17.61	6.23	2.35	5.26	Normal plant.
S*-1	0.02% MMS	48.49	61.34	116.82	17.59	6.39	2.83	5.69	Increased height, fruits length, fruit girth and improved yield
S-2	0.02% MMS	48.49	67.41	116.53	19.11	6.21	2.44	5.93	Tall, with increased number of fruits and subsequently increased yield.
S-3	0.03% MMS	48.49	58.43	97.00	17.59	6.19	2.31	5.30	Early maturing, normal plant with good yield.
S-4	0.03% MMS	44.76	39.87	117.73	11.39	4.73	1.84	4.26	Dwarf plant, poor branching and fruiting, smaller fruits, poor yield.
S-5	0.04% MMS	_	63.58	_	_	_	_	_	Tall, highly branched, vigorous but sterile plant.
S-7	0.03% DES	49.13	61.29	116.59	17.54	6.89	2.38	6.10	Tall, larger fruit and improved yield.
S-8	0.03% DES	47.76	44.77	115.10	20.00	6.68	2.51	6.14	Semi dwarf, long thick fruit, increased yield.
S-9	0.03% DES	55.27	58.11	128.44	19.45	6.31	2.38	5.96	Late maturing, with increased no. of fruits and yield.
S-10	0.04% DES	48.70	39.54	115.29	16.22	6.44	1.73	4.77	Dwarf, long thin fruit with reduced yield.

S*=Selection.

Table 27: Selected Mutants in Variety G4 (M_2 Generation).

Code	Treatments	Days to-flowering	Plant-height	Days to maturity	Fruit/plant	Fruit-length (cm)	Fruit girth (cm)	Total yield/plant (g)	Important Characters
-	Control	48.78	58.48	116.62	17.61	6.23	2.35	5.26	Normal plant.
S*-1	0.02% MMS	48.49	62.34	116.82	17.59	5.90	2.83	5.30	Tall, short fruit with normal yield
S-2	0.02% MMS	48.49	59.41	116.53	19.11	6.45	2.44	5.93	Long slender and thick fruit and increased yield.
S-3	0.03% MMS	48.49	63.43	97.00	17.59	6.19	2.31	6.10	Tall, Early maturing, normal plant with improved yield.
S-4	0.04% MMS	44.76	39.87	117.73	11.39	4.73	1.84	4.26	Dwarf plant with normal fruit but aborted seeds.
S-5	0.02% DES	47.10	57.85	116.55	19.30	6.20	2.30	5.95	Vigorous, increased no. of fruits and yield.
S-6	0.03% DES	49.13	45.15	116.59	17.54	6.89	2.38	6.10	Semi dwarf, larger fruit and improved yield.
S-7	0.03% DES	47.76	48.77	115.10	20.00	6.68	2.51	6.14	Normal height, late maturing, thick fruit and increased yield.

4.11 STUDIES IN M_3 GENERATION:

4.11.1 Seed germination:

In M_3 generation the percentage of seed germination decreased with increasing concentrations of each mutagen in both the varieties. The maximum reduction in germination was observed at the highest concentration of both the mutagens. The seed germination in control plant of var. Pusa jwala was 94.20%. It decreased to 60.27% in 0.05% MMS and 63.20% in 0.05% DES respectively in var. Pusa jwala (Table 28). In var. G4 the seed germination in control was 91.34%. However, the seed germination was decreased to 63.81% in 0.05% MMS and 66.58% in 0.05% DES (Table 29). Although, there was a decreasing trend in seed germination with the increasing concentration of both the mutagens but considerable recovery was noted in M_3 generation in comparison to M_1 & M_2 generation.

4.11.2 Plant Survival:

The survival of plants decreased with an increase in concentration of both the mutagens in both varieties viz. Pusa jwala and G4, (Table 28&29). The maximum survival percentage was observed at the lowest concentration and decreased linearly in higher concentration of both mutagens from 93.15% (control) to 64.35% in 0.05% MMS and 66.23% in 0.05% DES in var. Pusa jwala (Table 28). In var. G4 it was reduced from 91.35% (control) to 70.11% in 0.05% MMS and 72.39% in 0.05% DES (Table29).The highest lethality (30.91% and 23.25%) were recorded at 0.05% MMS in the var. Pusa jwala and G4 respectively (Table 28&29). Pusa jwala was found to be more sensitive than var. G4 as the survival in Pusa jwala was more adversely affected by mutagens and that MMS was found to be more affective in inducing lethality.

4.11.3 Pollen Fertility:

The pollen fertility in control plants was 90.63% and 91.36% in Pusa jwala and G4 respectively. It decreased from 85.64 – 71.88% in 0.01 – 0.05% MMS and 87.13 – 73.19

% in 0.01 – 0.05% DES in var. Pusa jwala (Table 28), while in var. G4 it ranged between 85.11–70.11% and 88.23–73.83% in 0.01-0.05% MMS and DES respectively (Table 29). Reduction in pollen fertility was found maximum (20.68%) in 0.05% MMS followed by (19.24%) in 0.05% DES in var. Pusa jwala while in var. G4 it was maximum (23.25.15%) in 0.05% MMS followed by (19.18%) in 0.05% DES. The MMS was highly effective on pollen fertility as compared to DES in both varieties. However, pollen fertility in var. Pusa jwala was more affected (Table 28&29).

4.11.4 Mutation frequency:

The mutations at maturity of the plants were found to increase with increasing concentrations of both the mutagens. In control populations of both varieties the variations were absent. In var. Pusa jwala it increased from 1.11 – 2.95% and 0.94 – 2.14% in 0.01 – 0.05% MMS and DES respectively (Table 28). The mutation frequency in var. G4 was increased from 1.02 – 2.09% and 0.78 – 1.87% in 0.01 – 0.05% MMS and DES respectively (Table 29). The variations, which were noted at older plant stage in M_2 generation such as stunted/improved growth, improved/poor branching and fruiting, increased/reduced fruit size etc. were inherited in M_3 also, but the frequency was lesser than M_1&M_2 generation.

4.12 MEIOTIC STUDIES IN M_3 GENERATION:

Cytological abnormalities were studied in M_3 generation also. The frequency of abnormalities was found to be lesser in comparison to M_1 and M_2 generations. The frequency of various meiotic aberrations in each variety treated with both the mutagen are presented in Tables 30 – 32. Various meiotic abnormalities at different stages of meiosis were recorded as follows.

4.12.1 Metaphase-I/II:

The meiotic aberrations recorded at metaphase-I/II showed a dose dependent increase in treated populations and it increased from 0.79 – 4.42% in 0.01 – 0.05% MMS and 0.81– 4.34% in 0.01 - 0.05% DES in var. Pusa jwala (Table 30). In var. G4 the number of

abnormal cells increased from 1.75 – 5.02% in 0.01 – 0.05% MMS and 0.77– 4.46% in 0.01 – 0.05% DES (Table 31).

4.12.2 Anaphase-I/II:

A dose dependent increase in the frequency of pollen mother cells showing meiotic abnormalities at anaphase-I/II was observed in both the varieties. In var. Pusa jwala it increased from 1.07 – 4.04% in 0.01 – 0.05%MMS and 1.01 – 3.72% in 0.01 – 0.05%DES (Table 30). In var. G4 also, meiotic aberration increased from 0.83 – 3.83% in 0.01 – 0.05%MMS and from 0.39 – 3.24% in 0.01 – 0.05%DES (Table 31).

4.12.3 Telophase-I/II:

At telophase-I/II also the meiotic aberrations were found to be dose dependent and increased with an increase in concentration of both mutagens in both the varieties. In var. Pusa jwala it increased from 0.94 – 2.82% in 0.01 – 0.05% MMS and from 0.82 – 1.96% in 0.01 – 0.05% DES (Table 30). In var.G4 also it increase from 0.88 – 3.21% in 0.01 – 0.05% MMS and from 0.30 – 2.63 in 0.01 – 0.05% DES (Table 31).

Perusal of the results in presented in Table 11, 20 and 32 revealed that meiotic aberrations increased with the increase in the concentrations of each mutagen in both the varieties. The frequency of meiotic aberrations was comparatively more in var.G4 than var. Pusa jwala. Moreover, the frequency of meiotic aberrations recorded in M_2 & M_3 generation was less than M_1 and the order of meiotic aberrations in M_1, M_2 & M_3 was $M_1 > M_2 > M_3$.

4.13 Chiasma frequency per cell and per bivalent at Metaphase-I:

The chiasma frequency at metaphase-I was decreased with an increase in concentration of both mutagens in var. Pusa jwala. In control plants the chiasma frequency was 18.96 per cell and 1.58 per bivalent. It decreased from 18.67 to 15.93 per cell and 1.56to 1.33 per bivalent in 0.01% - 0.05% MMS treatments while in 0.01% - 0.05% DES treatments it decreased from 18.89 to 16.35 per cell and 1.58 to 1.37 per

bivalent (Table 33). In var. G4 chiasma frequency in control plants was recorded as 18.84 and 1.57 per cell and per bivalent respectively. In 0.01 – 0.05% MMS treatment it reduced from 18.41 to 14.53 per cell and 1.53 to 1.22 per bivalent while in 0.01% – 0.05% DES treatments it reduced from 18.63 to 14.83 per cell and 1.55 to 1.23 per bivalent (Table 33).

4.14 QUANTITATIVE CHARACTERS:

The effect of MMS and DES was studied in M_3 generation also on seven quantitative traits viz., days to flowering, days to maturity, plant height (cm), number of fruits per plant, fruit length (cm), fruit girth (cm) and total yield per plant (g). Detail observations made for treated populations as well as control showing different parameters are presented in Tables 34 & 35.

4.14.1 Days to flowering and days to maturity:

The mean values for days to flowering was slightly decreased in lower concentrations of MMS and DES in both the varieties (Tables 34 & 35). However, there was a positive shift in the mean values toward control as compared to M_2 generation in both the varieties. Days to maturity was also increased significantly in higher concentrations of both the mutagen in both the varieties. The mean values for days to maturity was decreased in M_3 generation as compared to M_1 & M_2 generation i.e., positively shifted towards control (Tables 34 & 35).

4.14.2 Height of Mature Plants:

The height of mature plant followed the same trend as in M_1& M_2 generation and decreased in all the concentrations of both the mutagen in both the varieties (Tables 34 & 35). However, plant height also positively shifted towards control in M_3 generation. The order of mean values of plant height in M_1, M_2 and M_3 along with control was Control > $M_3 > M_2 > M_1$ (Tables 13, 14, 22, 23, 34 & 35).

4.14.3 Number of fruit/plant, fruit size and total yield per plant:

In var. Pusa jwala the mean values for fruit/plant and fruit size decreased in all the concentrations of both the mutagens except in 0.02%MMS and 0.03% DES where these values increased over the control (Tables 34). The yield per plant was also increased at 5% level in population treated with these two concentrations. However, the yield per plant was more in population treated with 0.03% DES than 0.02%MMS. In var. G4 also the number of fruits/plant, fruit size and total yield per plant were decreased in higher concentrations of both the mutagen while lower concentration (0.02%MMS and 0.03% DES) showed stimulatory effect and subsequently the yield was increased at 5% level in population treated with both the concentrations (Tables 35).

4.15 SELECTED MUTANTS IN M_3 GENERATION:

Ten promising mutant were isolated from M_3 generation of both the varieties (Table 36&37). A brief description of the mutants is as follows.

4.15.1 Mutants in Var. Pusa jwala.

In var. Pusa jwala six mutants were isolated in different concentrations of MMS and DES (Table 36).

Selection-I Highly branched with long thin fruit and improved yield (0.02%MMS, Plate-VII, fig. a&b).

Selection-II Tall with increased number of fruit and improved yield (0.02%MMS, Plate-VIII, fig. a&b).

Selection-III Long thick fruits and improved yield (0.02%DES, Plate-IX, fig. a, b &c).

Selection-IV Short thick fruit with improved yield (0.03%DES, Plate-X, fig. a, b &c).

Selection-V Large number of fruits and high yielding (0.03%DES, Plate-XI, fig. a&b).

Selection-VI Early maturing mutant (0.03%MMS, Plate-XII, fig. a&b)

4.15.2 Mutants in G4.

In var. G4 four mutants were isolated in different concentrations of MMS and DES (Table 37).

Selection-I	Medium size plant with long fruits and improved yield (0.02%MMS, Plate-XIV, fig. a&b).
Selection-II	Highly branched, short fruits and improved yield (0.02%MMS, Plate-XV, fig. a&b).
Selection-III	Tall and high yielding plant (0.03%DES, Plate- XVI, fig. a&b).
Selection-IV	Dwarf plant with early maturing fruits (0.02%DES, XVII, fig. a&b).

4.16 Capsaicin content of high yielding mutant:

Capsaicin ($C_{18}H_{27}NO_3$) is the active ingredient of hot chili pepper which is not only used as spice to foods, but can cause the body to heat up, promoting expenditure of calories. Chemically it is a fat soluble phenolic compound which imparts pungent taste even if it is diluted to one part in eleven million parts of water. It is an odorless white crystal with severe burning pungency. One part in 100,000 can be detected by tasting. It has a molecular weight of 305.4118 g/mol, slightly soluble in carbon disulfide, hot water, practically insoluble in water, freely soluble in alcohol, ether, benzene and chloroform. It is fairly resistant to acids and alkali solutions at room temperatures.

The capsaicin content of all high yielding mutants isolated from varieties Pusa jwala and G4 are shown in Table 36 & 37. The HPLC chromatogram of capsaicin standard, control of both the varieties and of the mutants are shown in Fig. 1-11 and plate 18-123. The data obtained showed varying content of capsaicin among the isolated high yielding mutants in both the varieties. In var. Pusa jwala, the capsaicin content in control was 52.38% while in Selection 1-5 (high yielding mutants) it was 69.12, 69.12, 50.50, 33.57 and 91.40% respectively, while in var. G4 the control showed 29.23% capsaicin and the in selection 1-3 (high yielding) the capsaicin content was recorded as 49.53, 68.71 and 14.97% (Table 37).

Table 28. Effect of MMS and DES on seed germination, mutation frequency, plant survival and pollen fertility in M_3 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatment (%)	Germination (%)	Inhibition (%)	Mutation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	94.20	-	-	93.15	-	90.63	-
MMS							
0.01	89.35	5.15	1.11	84.39	9.40	85.64	5.50
0.02	84.39	10.40	1.51	80.67	13.39	82.10	9.41
0.03	80.17	14.90	1.89	77.45	16.85	79.84	11.90
0.04	73.67	21.80	2.15	73.61	20.97	75.23	17.00
0.05	60.27	36.02	2.95	64.35	30.91	71.88	20.68
DES							
0.01	90.12	4.33	0.94	85.51	8.20	87.13	3.80
0.02	87.93	6.65	1.05	83.63	10.22	83.94	7.38
0.03	82.45	12.47	1.48	78.11	16.14	81.46	10.11
0.04	74.67	12.74	1.86	74.78	19.72	77.57	14.41
0.05	63.20	32.90	2.14	66.23	28.90	73.19	19.24

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Table 29. Effect of MMS and DES on seed germination, mutation frequency, plant survival and pollen fertility in M_3 generation of *Capsicum annuum* L. var. G_4.

Treatment (%)	Germination (%)	Inhibition (%)	Mutation frequency (%)	Plant Survival (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
Control	91.34	-	-	90.35	-	91.63	-
MMS							
0.01	90.11	1.34	1.02	85.76	6.11	85.11	6.84
0.02	88.23	3.40	1.43	81.39	10.90	81.94	10.31
0.03	87.44	8.65	1.77	79.92	12.51	79.18	13.34
0.04	74.67	18.25	1.93	74.67	18.25	74.33	18.64
0.05	63.81	30.46	2.09	70.11	23.26	70.11	23.25
DES							
0.01	89.61	1.90	0.78	87.13	4.61	88.23	5.42
0.02	88.54	3.06	1.23	82.54	9.70	85.66	6.24
0.03	84.33	7.68	1.53	80.10	12.31	81.11	11.22
0.04	76.84	15.88	1.63	75.76	17.06	78.54	14.04
0.05	66.58	27.10	1.87	72.39	20.75	73.83	19.18

Table 30. Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_3 generation of *Capsicum annuum* L. var. Pusa jwala

Conc.	Metaphase I/II						Anaphase I/II					Telophase I/II				Total abe.
	Uni.	Multi.	Stic.	Preco.	Stray.	Total	Lag.	Bri.	Uneq.	Non-syn.	Total	Micr.	Dist.	Cyto	Total	(%)
Control	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–
MMS(%)																
0.01	0.15	0.11	0.23	0.30	-	0.79	0.64	-	0.43	-	1.07	0.73	0.21	-	0.94	2.80
0.02	0.22	0.41	0.60	0.13	-	1.23	0.54	0.16	0.45	-	1.15	0.89	0.14	0.06	1.09	3.47
0.03	0.29	0.31	0.52	0.16	0.09	1.38	0.73	0.19	0.31	0.11	1.34	1.06	0.16	0.33	1.55	4.27
0.04	1.45	0.90	0.96	0.80	0.15	4.26	1.10	0.21	0.80	0.53	3.24	1.43	0.22	0.21	1.86	9.36
0.05	1.13	0.86	1.46	0.51	0.46	4.42	1.61	0.76	0.80	0.87	4.04	1.61	1.10	0.11	2.82	11.28
DES(%)																
0.01	0.12	0.17	0.33	0.19	-	0.81	0.54	0.16	0.31	-	1.01	0.63	0.13	0.06	0.82	2.64
0.02	0.26	0.34	0.46	0.10	-	1.61	0.56	0.27	0.27	-	1.10	0.55	0.21	0.33	1.09	3.35
0.03	0.35	0.22	0.61	0.21	-	1.39	0.44	0.21	0.54	-	1.19	0.80	0.08	0.23	1.11	3.69
0.04	1.09	1.16	0.71	0.71	0.13	3.84	0.93	0.22	0.89	0.76	2.79	1.10	0.40	0.30	1.80	8.43
0.05	1.11	1.40	0.79	0.79	0.93	4.34	1.13	0.90	1.06	0.63	3.72	1.06	0.61	0.29	1.96	9.70

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**=precocious separation, **Stray**.=stray bivalent, **Lag.**=laggard, **Bri.**=bridge, **Uneq.**=unequal separation, **Non-syn.** = Non-synchronization of bivalents/chromosome, **Micro.**=micronuclei, **Dist.**=disturbed polarity, **Cyto.**=cytomixis, **Total abe.**= total aberration, **-** = not observed.

Table 31: Abnormalities (%) at different stages of meiosis induced by MMS and DES in M_3 generation *Capsicum annuum* L. var. G4.

Conc.	Metaphase I/II					Total	Anaphase I/II			Total	Telophase I/II			Total	Total Abe. (%)
	Uni.	Multi.	Stic.	Preco.	Stray.		Lag.	Bri.	Uneq.		Micr.	Dist.	Cyto.		
Control	–	–	–	–	–		–	–	–	–	–	–	–	–	–
MMS(%)															
0.01	0.11	0.43	0.49	0.21	0.51	**1.75**	0.51	0.11	0.21	**0.83**	0.10	0.19	0.59	**0.88**	**3.46**
0.02	0.19	0.80	0.60	0.31	0.19	**2.09**	0.29	-	0.53	**0.93**	0.17	0.51	0.62	**1.30**	**4.32**
0.03	0.43	0.96	0.90	0.19	0.16	**2.64**	0.82	0.49	0.28	**1.59**	0.21	0.71	0.60	**1.52**	**5.75**
0.04	0.48	1.06	1.34	0.51	0.96	**4.35**	1.13	0.76	1.05	**2.94**	0.45	0.91	0.80	**2.16**	**9.45**
0.05	0.70	1.36	1.11	0.76	1.09	**5.02**	1.29	1.13	1.41	**3.83**	1.01	1.11	1.09	**3.21**	**12.06**
DES(%)															
0.01	0.13	0.17	0.34	0.10	0.13	**0.77**	0.10	0.12	0.17	**0.39**	0.11	0.13	0.06	**0.30**	**1.46**
0.02	0.19	0.49	0.54	0.26	0.46	**1.94**	0.48	0.11	0.19	0.78	0.17	0.31	0.14	**0.62**	**3.34**
0.03	0.51	0.73	0.81	0.16	0.41	**2.62**	0.76	0.13	0.51	**1.40**	0.63	0.59	0.07	**1.29**	**5.31**
0.04	0.61	0.86	1.13	0.51	0.81	**3.92**	1.10	0.53	1.11	**2.74**	0.86	0.70	0.45	**2.01**	**8.67**
0.05	0.82	1.06	1.17	0.65	0.76	**4.46**	1.23	0.87	1.14	**3.24**	1.33	0.70	0.53	**2.63**	10.33

Conc.=concentration, **Uni.**=univalent, **Multi.**=multivalent, **Stic.**=stickiness, **Preco.**= precocious separation, **Stray.**=stray bivalent, **Bri.**=bridge, **Lag.**=laggard, **Uneq.**=unequal separation, **Micro.**=micronuclei, **Dist.**=disturbed polarity, **Cyto.**=cytomixis, Total Abe.=Total Aberrations, - = not observed.

Table 32: Comparison of frequency of meiotic abnormalities induced by MMS and DES at different stages of meiosis in M_3 generation of *Capsicum annuum* L. var. Pusa jwala and G4.

Treatments	Var. Pusa jwala			Total Abnormalities (%)	Var. G4			Total Abnormalities (%)
	Metaphase I/II	Anaphase I/II	Telophase I/II		Metaphase I/II	Anaphase I/II	Telophase I/II	
Control	-	-	-	-	-	-	-	-
MMS (%)								
0.01	0.79	1.07	0.94	**2.80**	1.75	0.83	0.88	**3.46**
0.02	1.23	1.15	1.09	**3.47**	2.09	0.93	1.30	**4.32**
0.03	1.38	1.34	1.55	**4.27**	2.65	1.59	1.52	**5.75**
0.04	4.26	3.24	1.86	**9.36**	4.35	2.94	2.16	**9.45**
0.05	4.42	4.04	2.82	**11.28**	5.02	3.83	3.21	**12.06**
DES (%)								
0.01	0.81	1.01	0.82	**2.64**	0.77	0.39	0.30	**1.46**
0.02	1.61	1.10	1.09	**3.35**	1.94	0.78	0.62	**3.34**
0.03	1.39	1.19	1.11	**3.69**	2.62	1.40	1.29	**5.31**
0.04	3.84	2.79	1.82	**8.43**	3.92	2.74	2.01	**8.67**
0.05	4.34	3.72	1.96	**9.70**	4.46	3.24	2.63	**10.33**

Table 33- Effect of MMS and DES on chiasma frequency at Metaphase-I in M_3 generation of *Capsicum annuum* L. var. Pusa jwala and G_4.

Treatment	Var. Pusa jwala		Var. G_4	
	Chiasma/cell	Chiasma/bivalent	Chiasma/cell	Chiasma/bivalent
Control	18.96±0.20	1.58±0.06	18.84±0.17	1.57±0.08
MMS (%)				
0.01	18.67±0.16	1.56±0.06	18.41±0.18	1.53±0.08
0.02	18.38±0.17	1.54±0.09	17.49±0.13	1.46±0.06
0.03	17.59±0.13	1.47±0.08	16.97±0.15	1.42±0.07
0.04	16.89±0.18	1.40±0.06	15.77±0.14	1.32±0.09
0.05	15.93±0.19	1.33±0.07	14.53±0.17	1.22±0.09
DES (%)				
0.01	18.89±0.15	1.58±0.05	18.63±0.19	1.55±0.06
0.02	18.59±0.19	1.55±0.07	17.77±0.21	1.49±0.06
0.03	17.88±0.21	1.50±0.08	17.25±0.22	1.43±0.08
0.04	16.97±0.20	1.42±0.08	15.96±0.19	1.33±0.07
0.05	16.35±0.18	1.37±0.07	14.83±0.23	1.23±0.09

Table 34: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_3 generation of *Capsicum annuum* L. var. Pusa jwala.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	49.31±0.51(7.39)	59.34±0.30(1.5[illegible])	116.33±0.29(1.31)	17.84±0.22(12.51)	6.29±0.19(10.62)	2.41±0.33(8.10)	5.38±0.12(6.77)
MMS(%)							
0.01	49.11±0.56(9.23)	58.84±0.39(2.43)	116.02±0.29(1.40)	17.11±0.41(11.34)	6.21±0.15(11.13)	2.30±0.11(12.00)	5.24±0.17(8.12)
0.02	49.23±0.63(10.29)	57.92±0.33(2.90)	116.23±0.44(1.51)	18.20±0.50(12.43)	7.13±0.21(12.10)	2.35±0.15(12.41)	5.96*±0.23(10.00)
0.03	49.79±0.59(10.87)	56.73*±0.37(3.49)	117.31±0.39(1.59)	17.01*±0.44(14.51)	6.15±0.32(16.10)	2.13±0.20(14.10)	5.21±0.15(10.77)
0.04	50.13*±0.70(11.15)	56.19**±0.51(4.10)	120.60*±0.51(2.11)	16.84*±0.52(16.36)	6.06±0.38(17.34)	1.90±0.31(15.70)	5.00±0.22(12.50)
0.05	51.23**±0.73(12.23)	53.41**±0.59(5.74)	122.67**±0.50(2.91)	15.72**±0.56(19.34)	5.94**±0.44(18.78)	1.84±0.29(17.64)	4.39**±0.31(13.61)
LSD at 5%	1.42*	2.13*	3.10*	1.12*	0.94*	NS	0.51*
LSD at 1%	2.49**	3.13**	4.05**	1.65**	1.33**	NS	0.89**
DFS(%)							
0.01	49.11±0.45(8.78)	59.11±0.41(3.05)	116.21±0.20(1.63)	17.33±0.49(12.17)	6.21±0.17(11.19)	2.36±0.19(9.61)	5.17±0.11(9.12)
0.02	48.97±0.52(9.07)	58.83±0.49(3.27)	116.28±0.30(1.55)	16.74±0.62(12.93)	6.15±0.22(13.21)	2.19±0.22(11.12)	5.10±0.19(11.00)
0.03	49.34±0.53(9.64)	57.79±0.56(3.93)	117.38±0.41(1.72)	19.88±0.54(12.83)	6.59±0.29(15.41)	2.77±0.29(13.23)	6.00*±0.27(14.27)
0.04	49.43*±0.61(10.4[illegible])	56.69*±0.51(5.11)	119.73*±0.42(1.35)	15.71*±0.61(15.55)	6.18±0.26(17.19)	2.20±0.26(15.70)	4.91±0.24(15.17)
0.05	50.11**±0.59(11.12)	54.94**±0.60(5.78)	121.22**±0.49(1.51)	13.44**±0.6(17.63)	5.36*±0.34(20.11)	2.02±0.23(15.60)	4.4[illegible]**±0.28(17.27)
LSD at 5%	1.22*	2.99*	3.17*	1.98*	0.88*	NS	0.57*
LSD at 1%	1.51**	4.11**	4.11**	2.70**	0.99**	NS	0.90**

Figures in Parenthesis represents CV%, S.E.– Standard Error, LSD– Least Significant difference.

Table 35: Estimates of Mean values ($\bar{x}$) and coefficient of variation (CV) for different quantitative characters in M_3 generation of *Capsicum annuum* L. var. G4.

Treatments	Days to flowering $\bar{x} \pm S.E$	Plant height $\bar{x} \pm S.E$	Days to maturity $\bar{x} \pm S.E$	Fruit/plant $\bar{x} \pm S.E$	Fruit length (cm) $\bar{x} \pm S.E$	Fruit girth (cm) $\bar{x} \pm S.E$	Total yield/plant (g) $\bar{x} \pm S.E$
Control	58.10±0.57(6.10)	60.92±0.39(1.72)	181.74±0.34(1.36)	14.23±0.72(11.33)	6.45±0.57(11.21)	2.87±0.18(10.21)	5.16±0.19(9.25)
MMS(%)							
0.01	57.96±0.51(6.07)	59.34±0.40(2.06)	181.60±0.44(1.41)	14.19±0.56(13.12)	6.11±0.40(11.13)	2.80±0.19(11.44)	5.13±0.24(10.31)
0.02	57.77±0.52(8.11)	57.92±0.51(2.16)	178.74±0.46(1.31)	15.10±0.50(14.02)	7.05±0.32(13.34)	2.94±0.22(12.33)	5.59*±0.20(11.16)
0.03	57.93±0.83(8.79)	56.84*±0.67(2.00)	179.67±0.67(1.51)	12.61*±0.59(15.11)	6.02±0.20(12.51)	2.72±0.26(15.24)	4.96±0.33(13.06)
0.04	59.28*±0.62(9.44)	53.29*±0.56(3.41)	180.10±0.61(2.33)	11.92**±0.60(17.77)	5.92±0.41(14.39)	2.57±0.26(18.21)	4.83±0.36(14.39)
0.05	62.54**±0.77(11.92)	51.78**±0.66(4.15)	181.23*±0.69(2.11)	1.44**±0.55(19.11)	5.33**±0.59(16.68)	2.43±0.36(21.11)	4.54**±0.35(17.13)
LSD at 5%	1.27*	4.77*	1.13*	1.33*	0.98*	NS	0.41*
LSD at 1%	2.66**	7.88**	1.94**	2.69**	1.22**	NS	0.63**
DES(%)							
0.01	57.89±0.34(7.11)	59.25±0.27(3.12)	181.54±0.50(1.41)	14.43±0.51(11.12)	6.34±0.11(11.19)	2.86±0.10(10.16)	5.15±0.11(9.12)
0.02	57.70±0.30(8.00)	58.72±0.34(3.71)	179.70±0.39(1.33)	12.93±0.62(14.21)	5.96±0.16(13.48)	2.80±0.16(12.12)	5.10±0.19(10.77)
0.03	57.98±0.35(9.13)	57.44±0.48(3.80)	180.93±0.46(1.44)	15.95±0.62(16.72)	6.99±0.22(16.39)	2.90±0.17(15.24)	5.66*±0.27(13.19)
0.04	58.43*±0.39(1.23)	56.43*±0.44(4.70)	182*.73±0.51(1.40)	12.33*±0.67(19.11)	5.48*±0.31(20.14)	2.69±0.26(18.12)	4.72±0.19(15.24)
0.05	62.20**±0.48(11.44)	52.73**±0.54(5.72)	183.11**±0.59(1.90)	10.51**±0.61(20.13)	5.10**±0.38(22.13)	2.39±0.30(21.10)	4.45**±0.26(17.67)
LSD at 5%	1.77*	5.11*	2.34*	2.13*	0.94*	NS	0.51*
LSD at 1%	3.19**	6.51**	2.96**	3.66**	1.39**	NS	0.73**

Figures in Parenthesis represents CV%, S.E. = Standard Error, LSD=Least Significant difference.

Table 36: Selected Mutants in var. Pusa jwala (M_3 Generation)

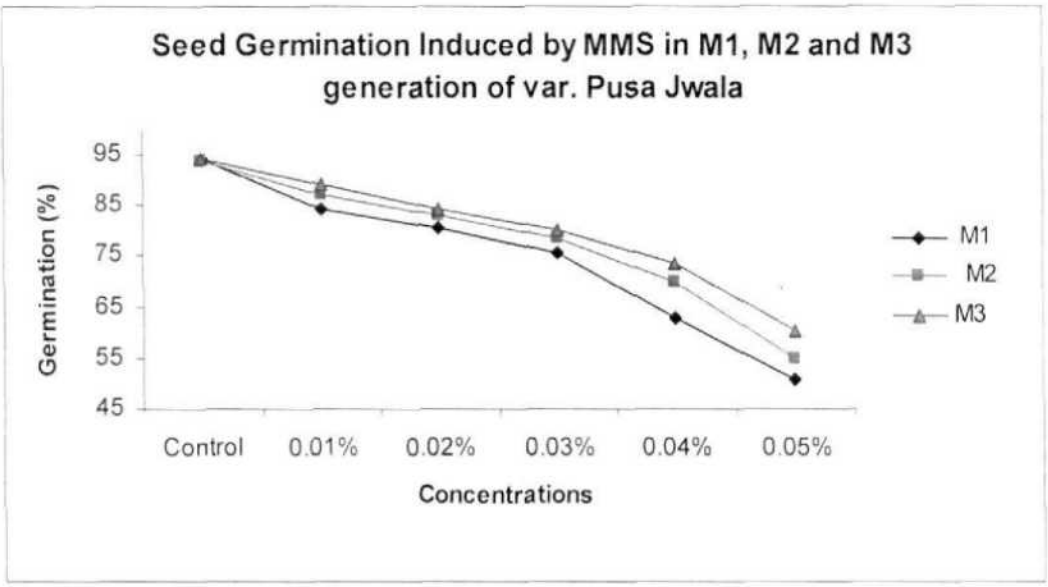
Code	Treatments	Days to-flowering	Plant-height	Days to maturity	Fruit/plant	Fruit-length (cm)	Fruit girth (cm)	Total yield/plant (g)	Capsaicin content (%)	Important Characters
-	Control	47.35	58.482	116.32	17.38	6.15	2.30	5.22	52.38	Normal plant showing good yield.
S*-I	0.02% MMS	48.10	58.52	116.33	17.29	6.50	2.15	5.93	69.12	Highly branched, long thin fruit and improved yield.
S*-II	0.02% MMS	47.86	65.71	116.10	20.22	6.17	2.26	6.25	50.50	Tall with increased number of fruit and improved yield.
S*-III	0.02% DES	47.10	58.36	116.80	17.41	6.53	2.44	6.71	33.57	Long thick fruits and improved yield.
S*-IV	0.03% DES	47.76	48.77	115.10	17.33	5.48	2.77	6.14	61.68	Short thick fruit improved yield.
S*-V	0.03% DES	49.27	58.36	116.80	21.45	6.13	2.36	6.33	91.40	Large number of fruits, improved yield.
S*-VI	0.03% MMS	46.33	58.10	102.10	17.35	6.12	2.31	5.23	-	Early maturing mutant.

S*= Selection.

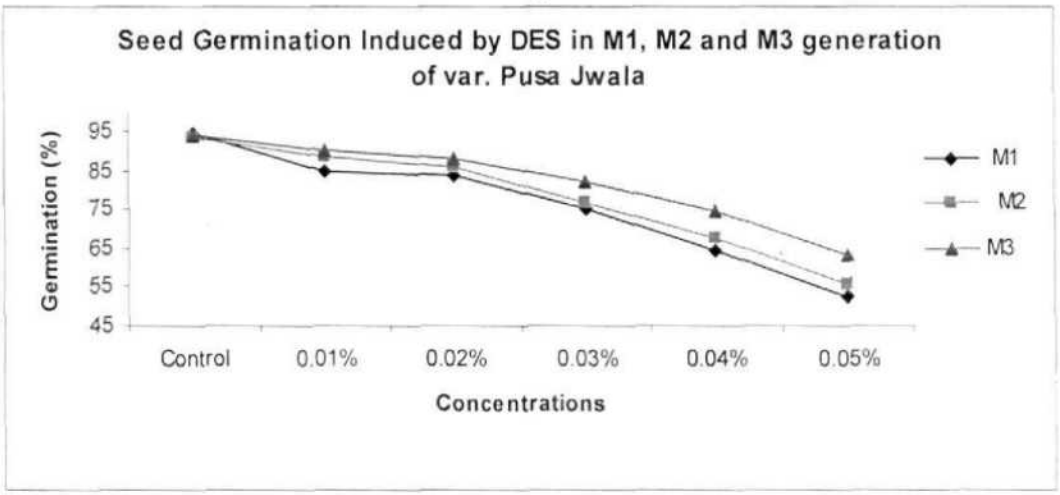
Table 37: Selected mutants in var. G4 (M_3 Generation)

Code	Treatments	Days to-flowering	Plant-height	Days to maturity	Fruit/ plant	Fruit Length (cm)	Fruit Girth (cm)	Total yield/ plant (g)	Capsaicin content (%)	Important Characters
-	Control	48.10	58.43	116.30	17.52	6.10	2.40	5.16	29.23	Normal plant showing good yield.
S*-I	0.02% MMS	48.36	58.25	116.20	17.65	5.11	2.39	6.24	49.53	Slightly reduced height, long fruit, improved yield
S*-II	0.02% MMS	47.12	48.30	115.82	17.67	7.52	2.49	5.95	68.71	Highly branched, short fruit and improved yield
S*-III	0.03% DES	48.15	69.44	116.62	23.40	6.16	2.38	6.52	14.97	Tall, Large number of fruit and improved yield
S*-IV	0.02% DES	43.31	58.44	96.27	17.49	6.20	2.35	5.23	-	Dwarf, Early maturing.

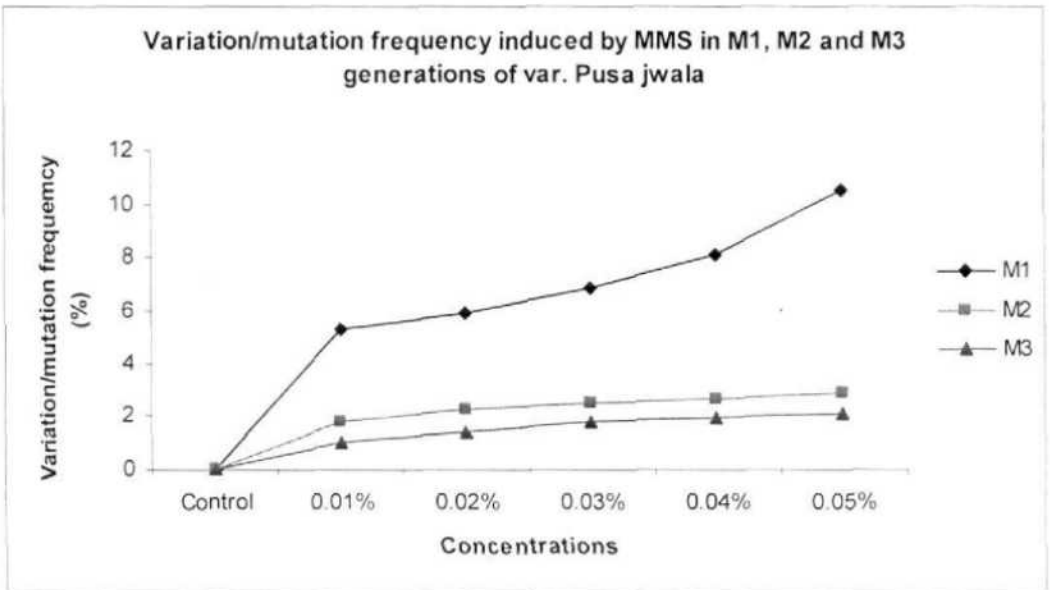
S* = Selection.



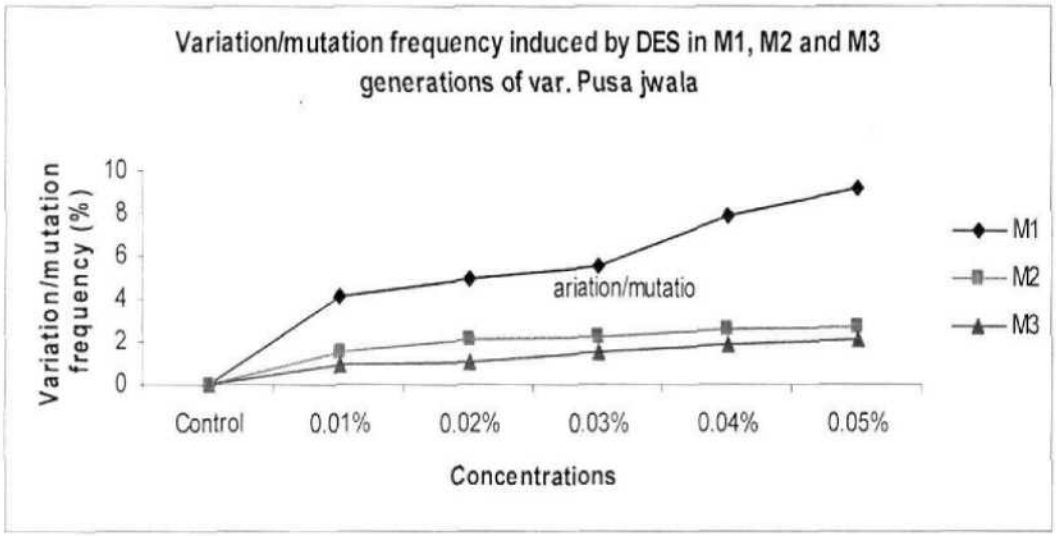
Graph - 1



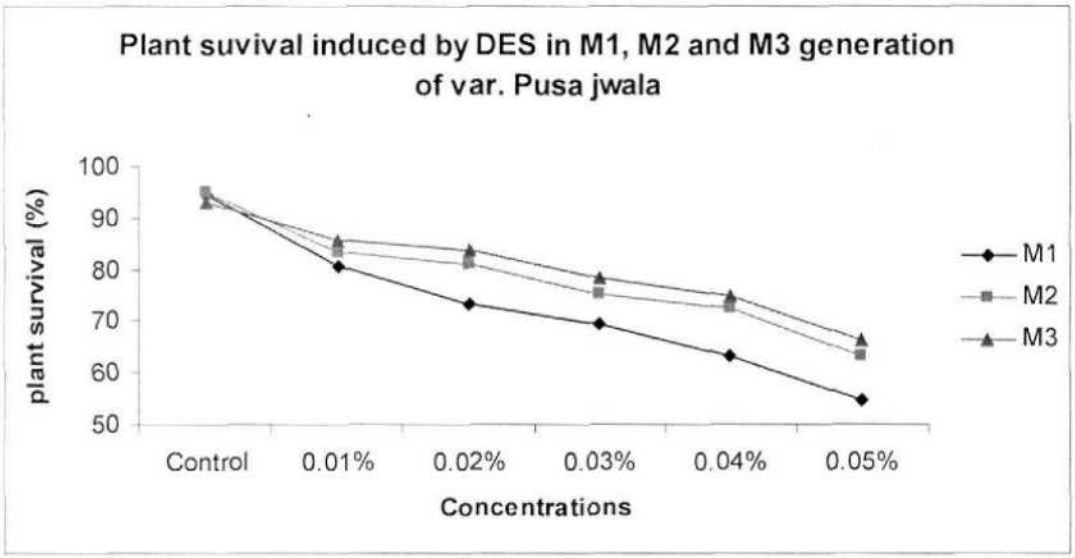
Graph - 2



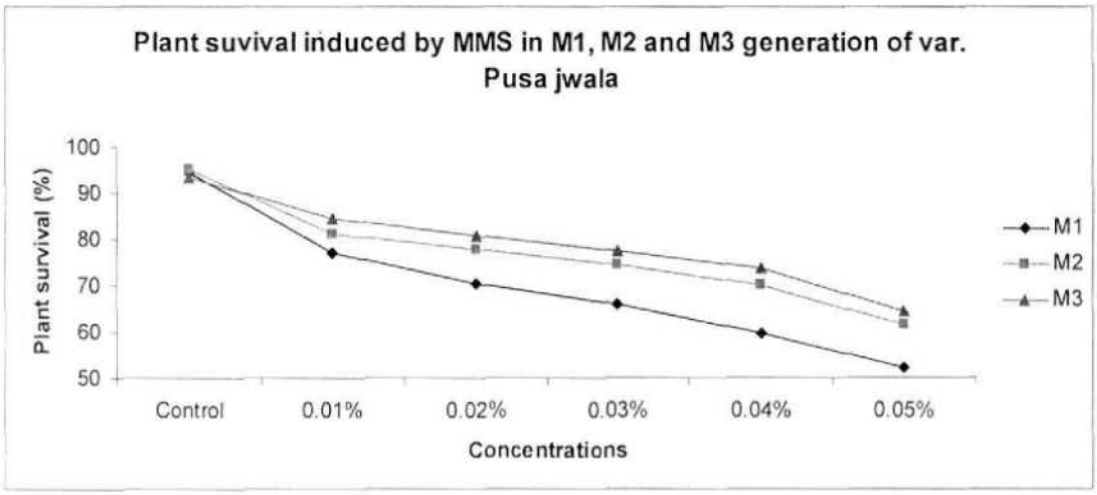
Graph - 3



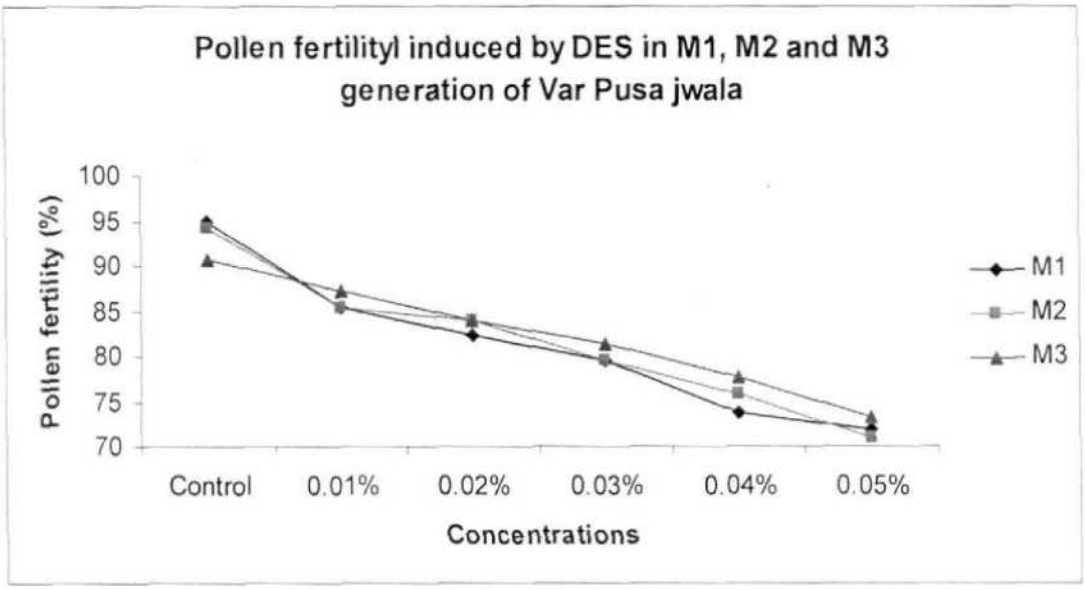
Graph - 4



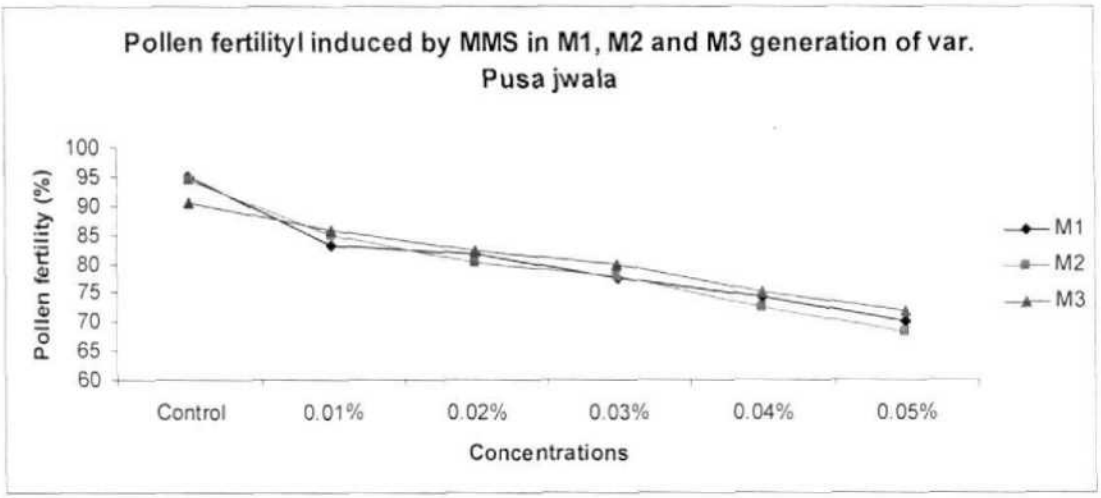
Graph - 5



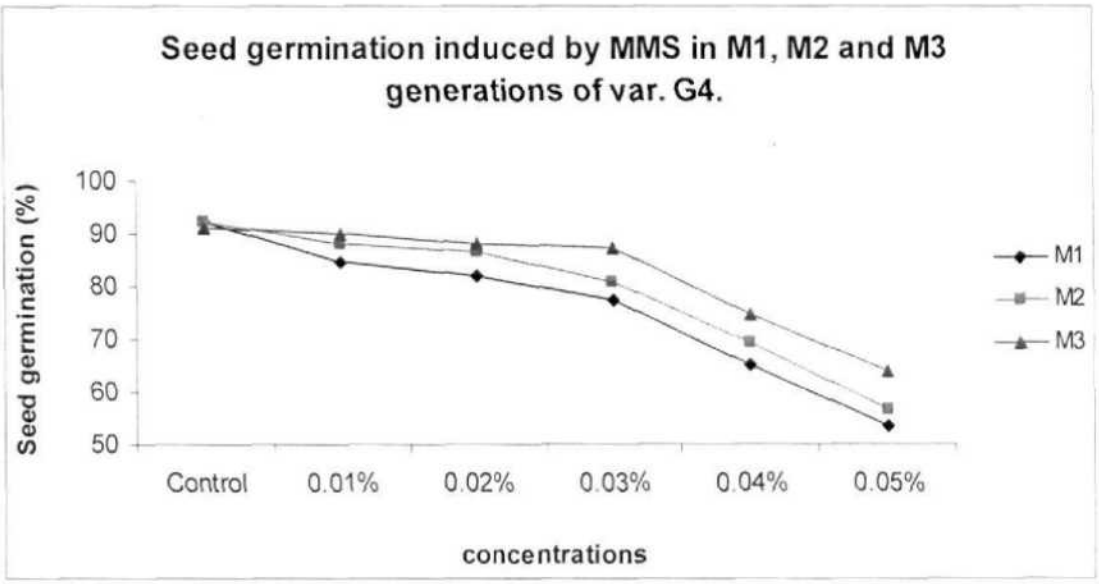
Graph – 6



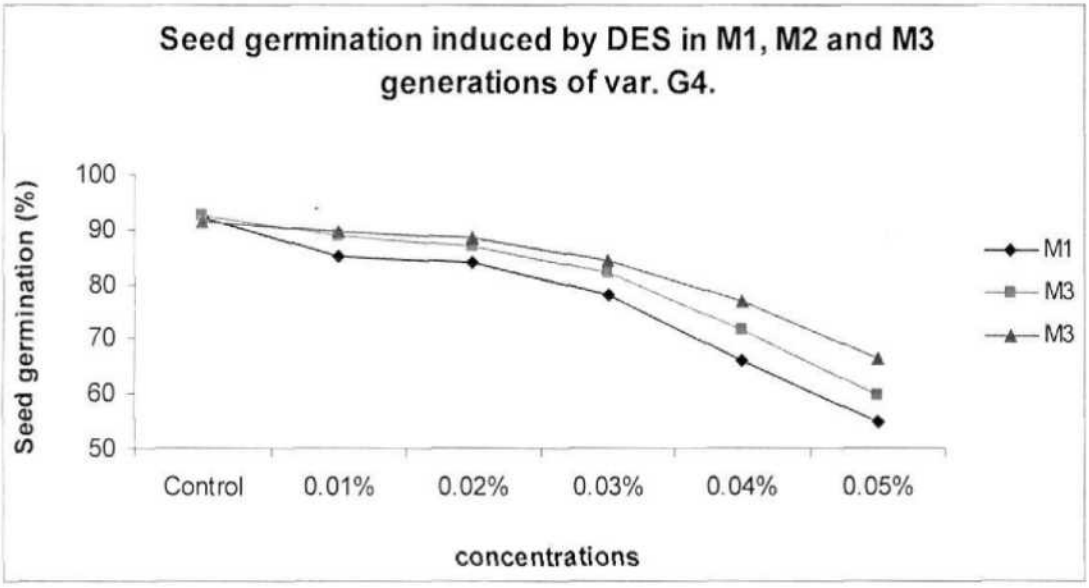
Graph – 7



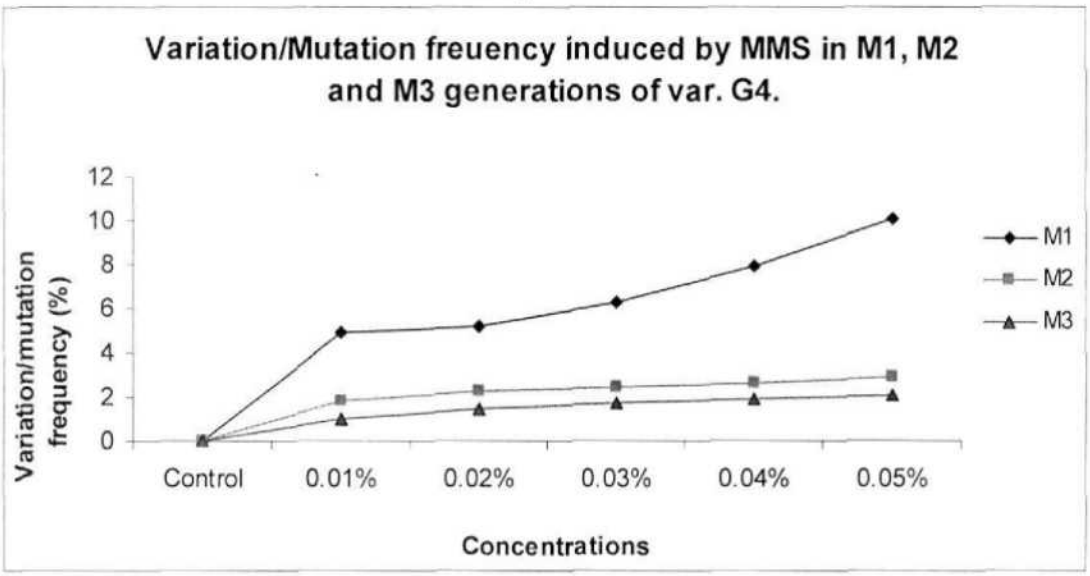
Graph – 8



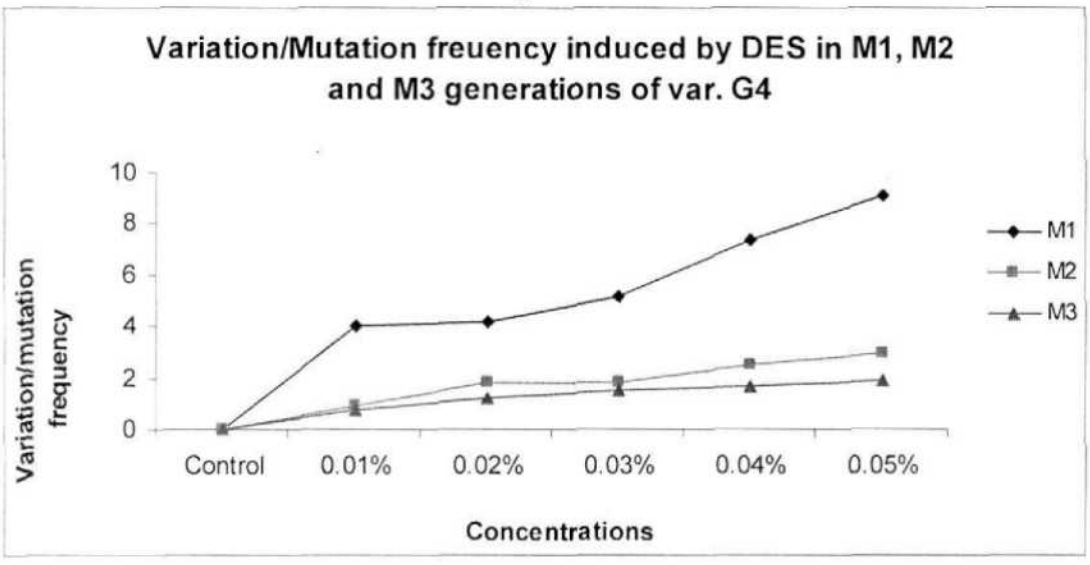
Graph – 9



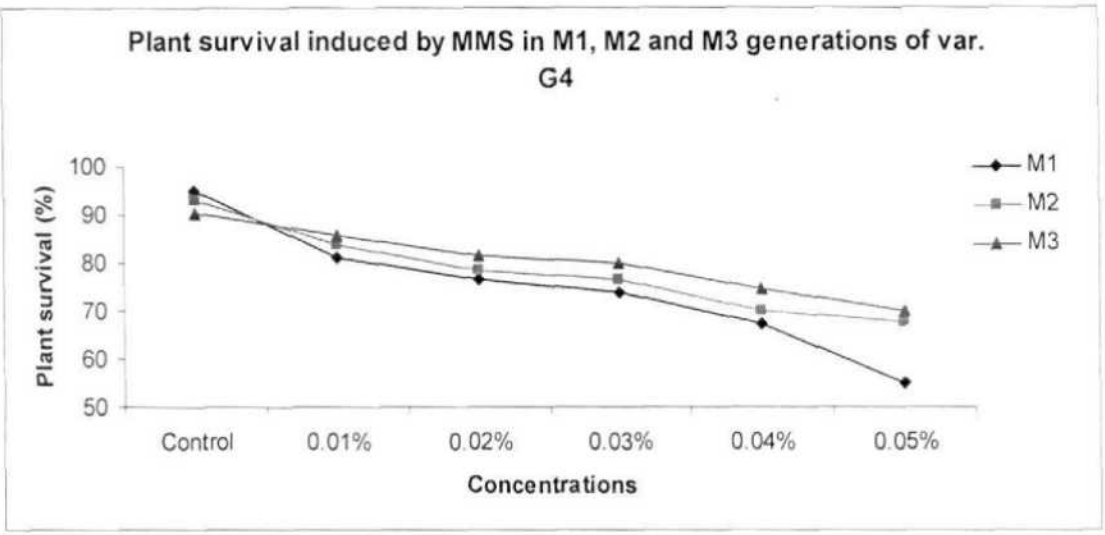
Graph – 10



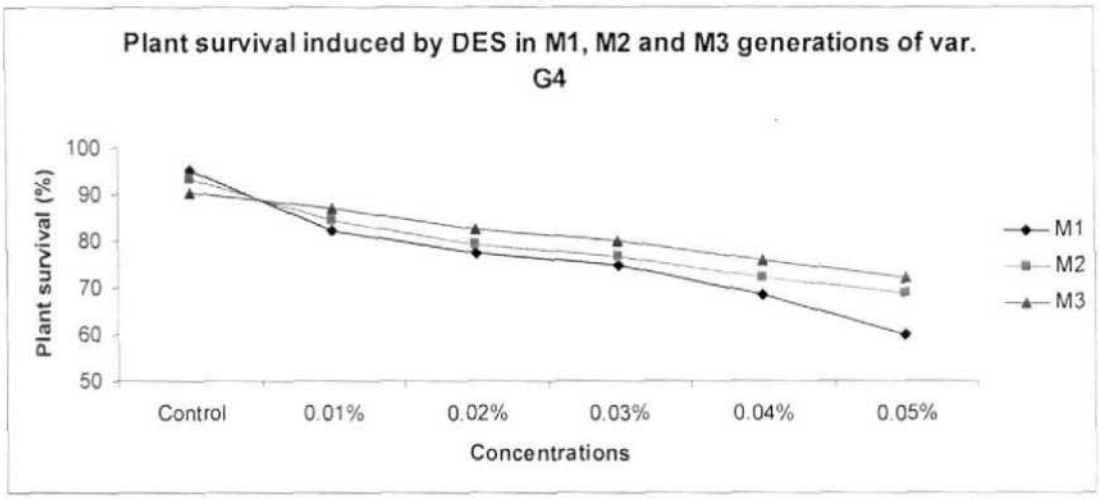
Graph – 11



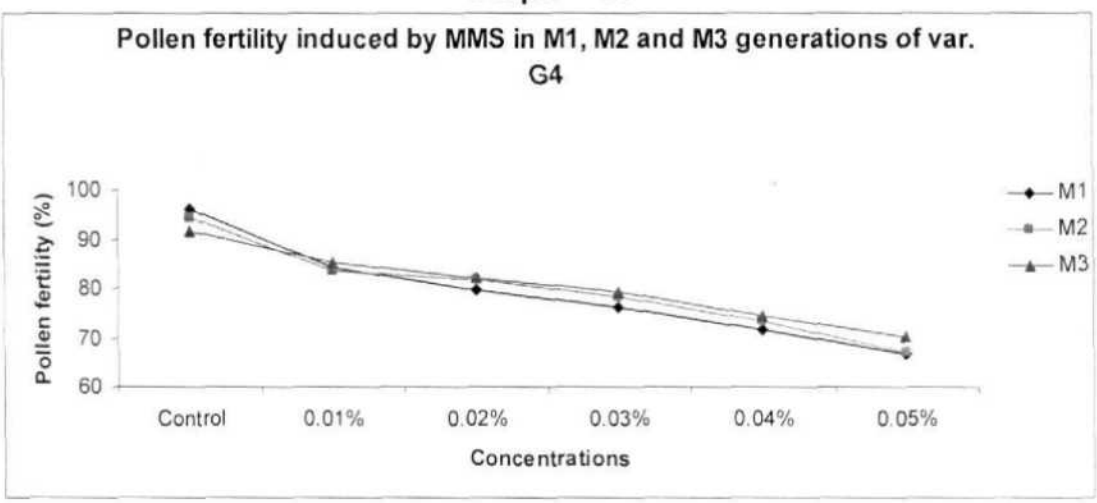
Graph – 12



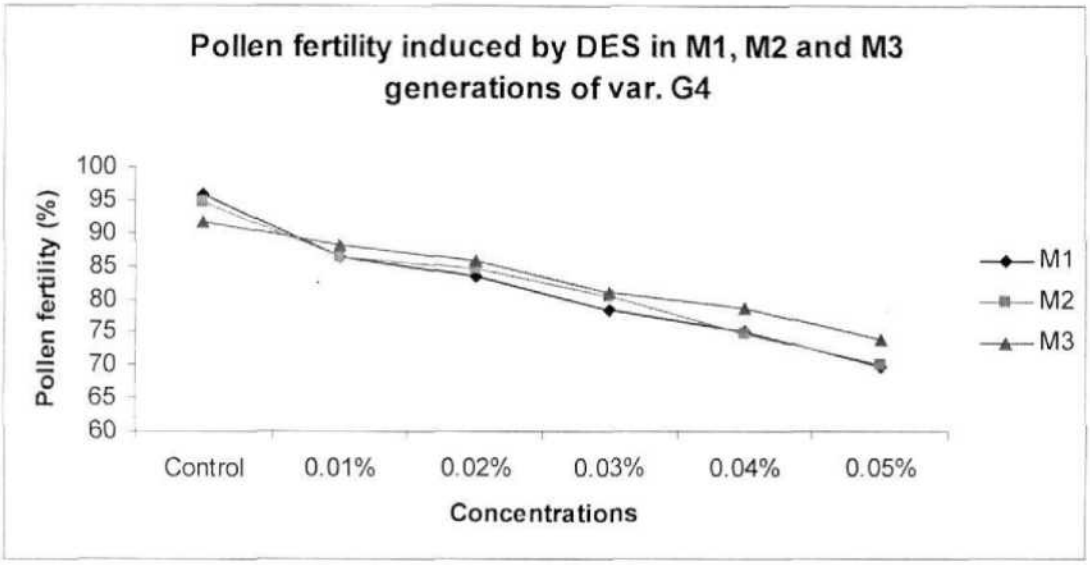
Graph – 13



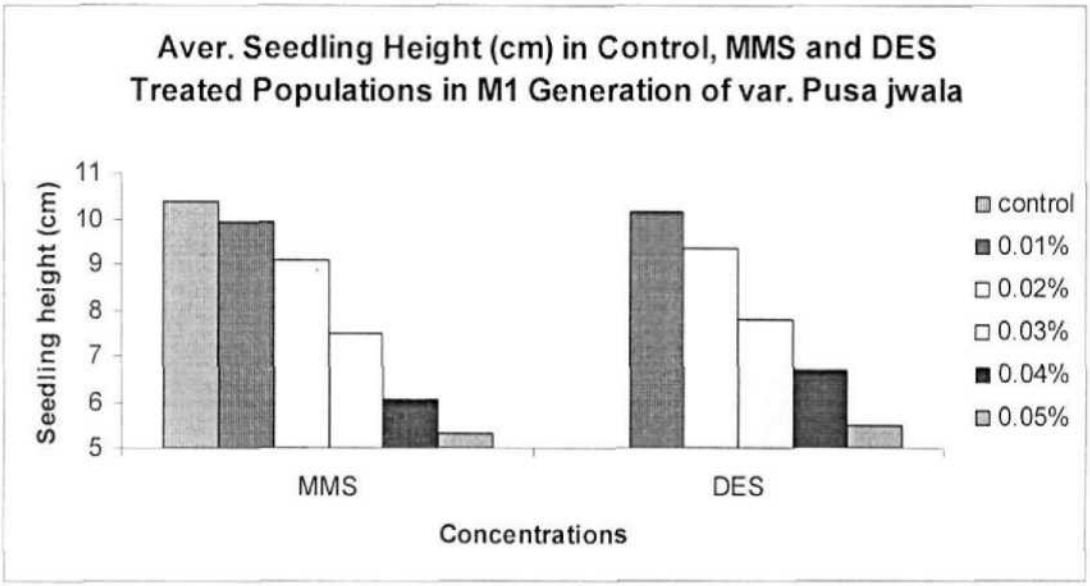
Graph – 14



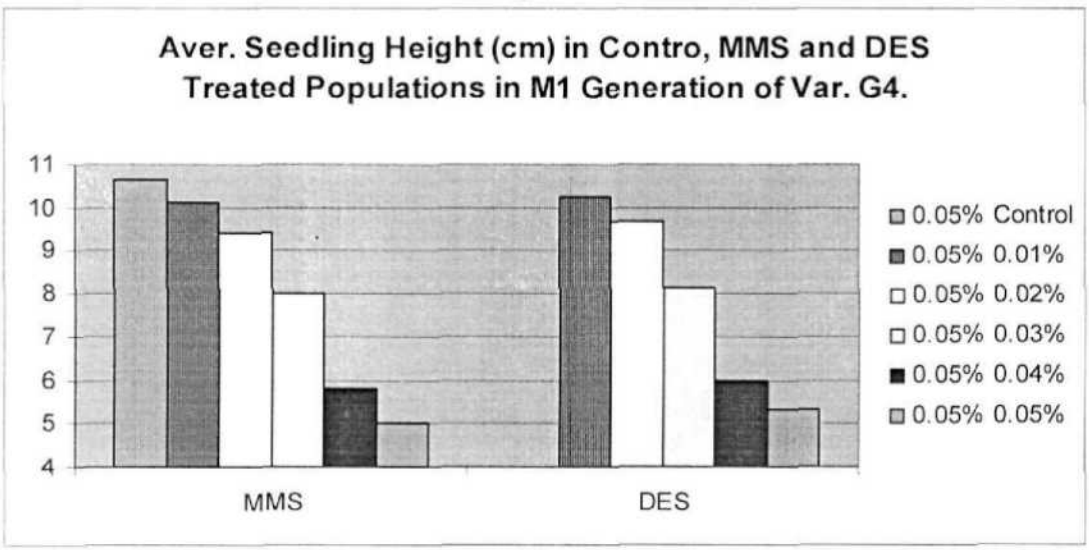
Graph – 15



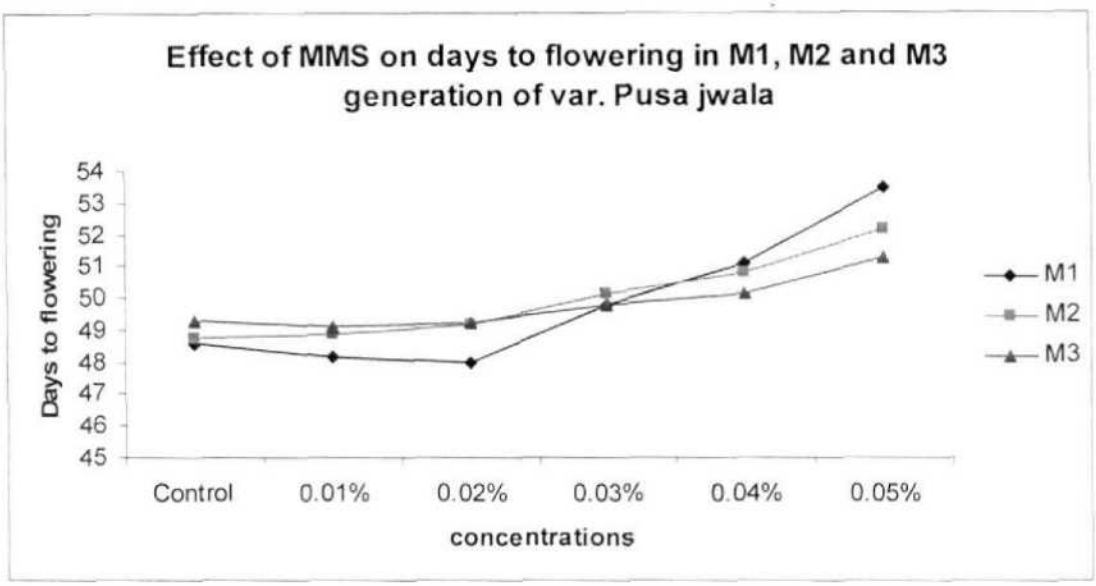
Graph – 16



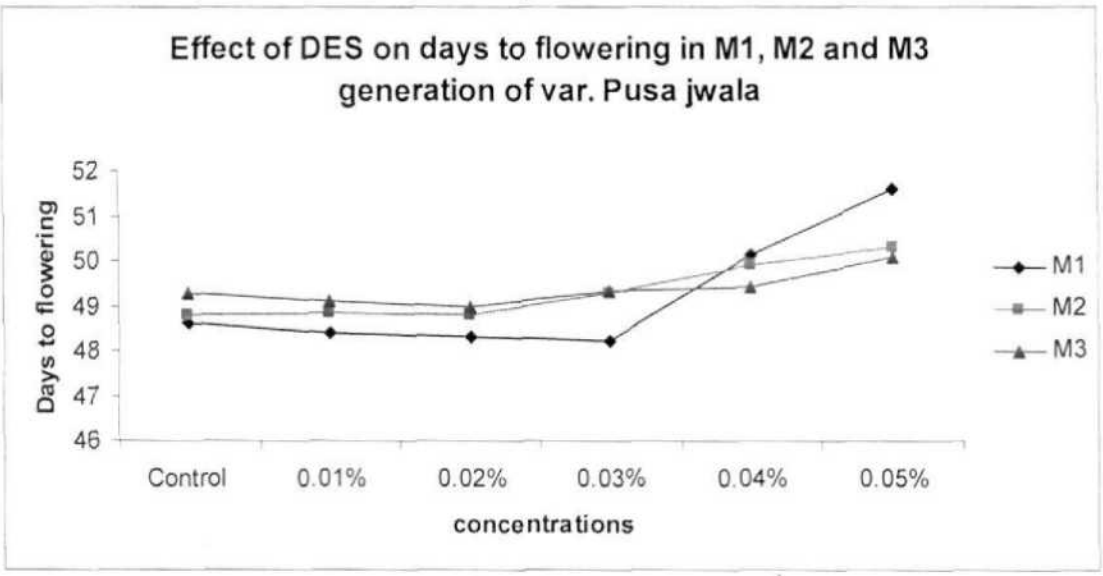
Graph – 17



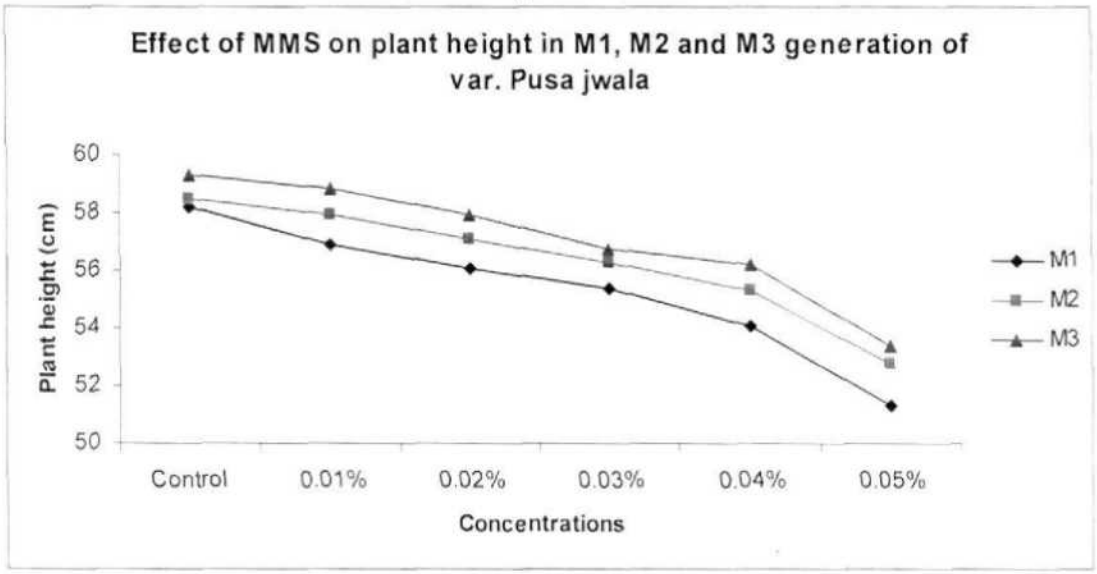
Graph – 18



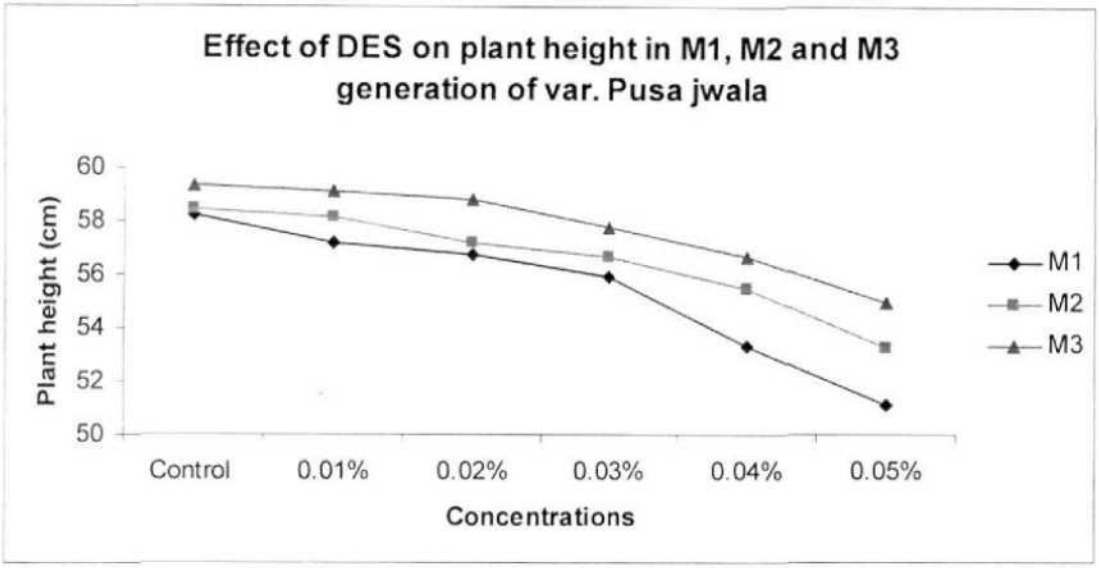
Graph – 19



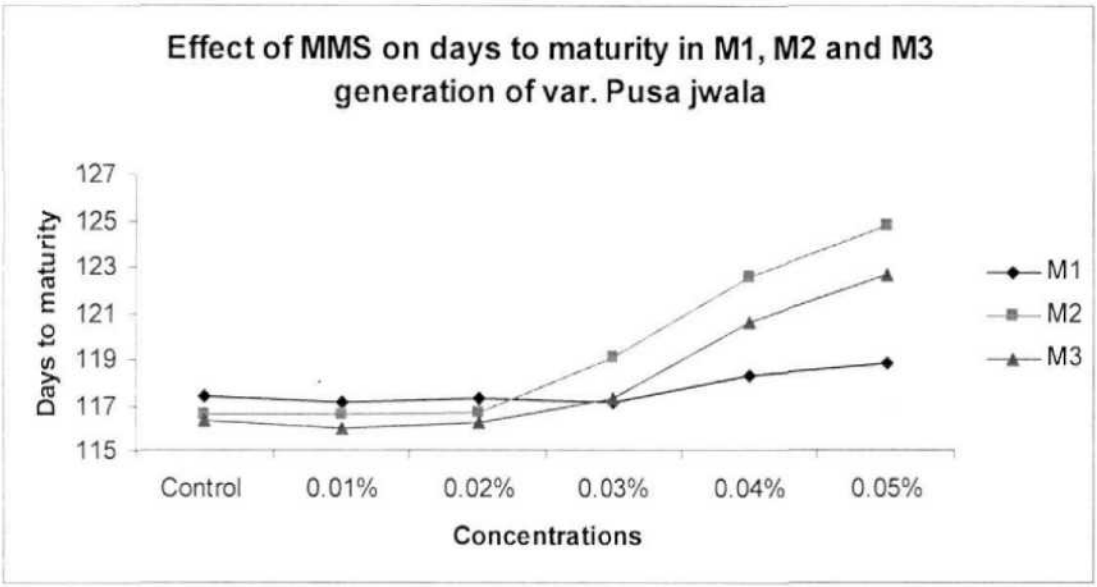
Graph – 20



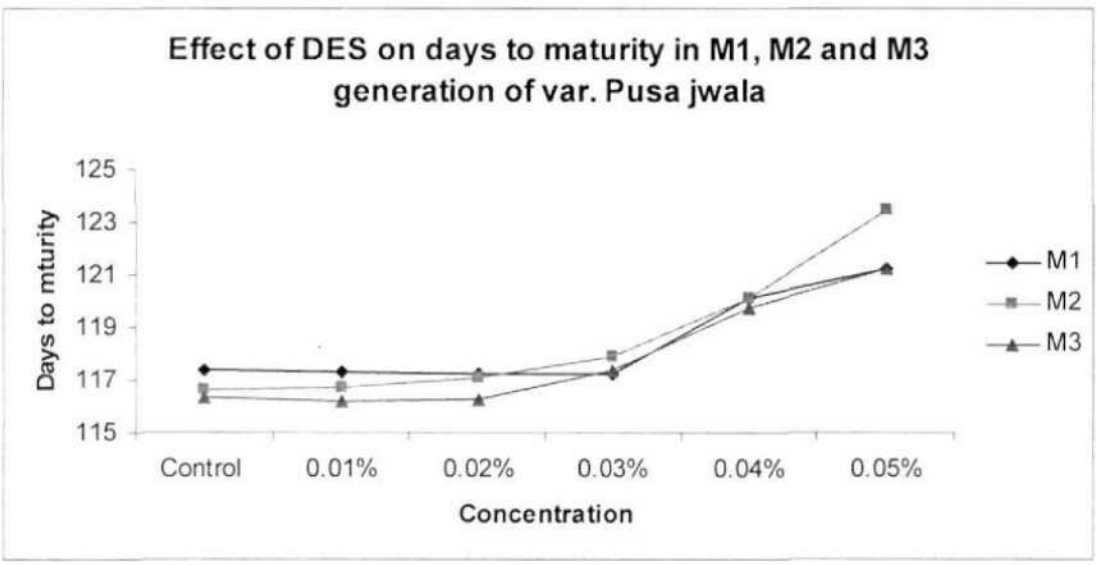
Graph – 21



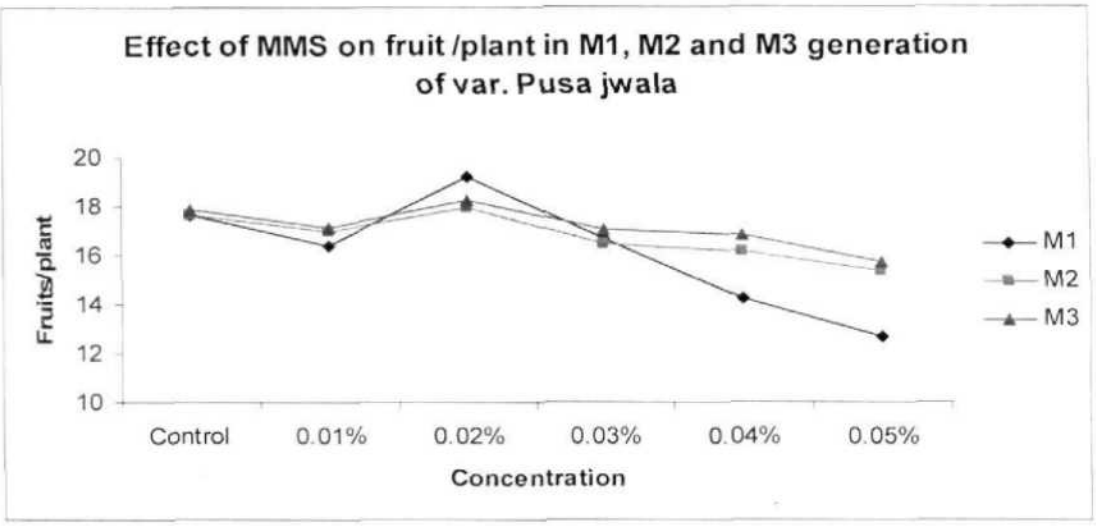
Graph – 22



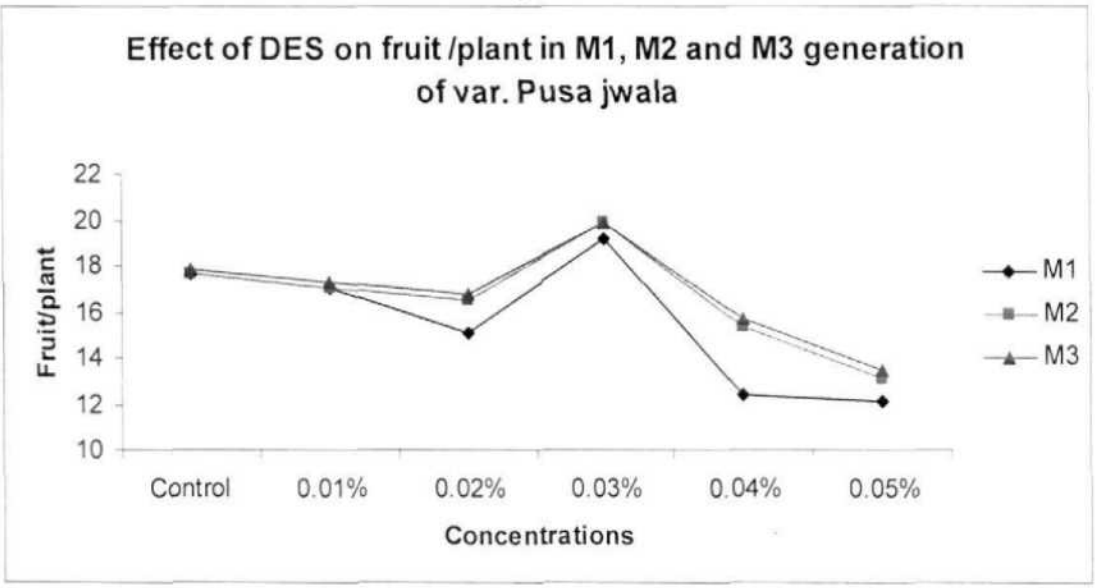
Graph – 23



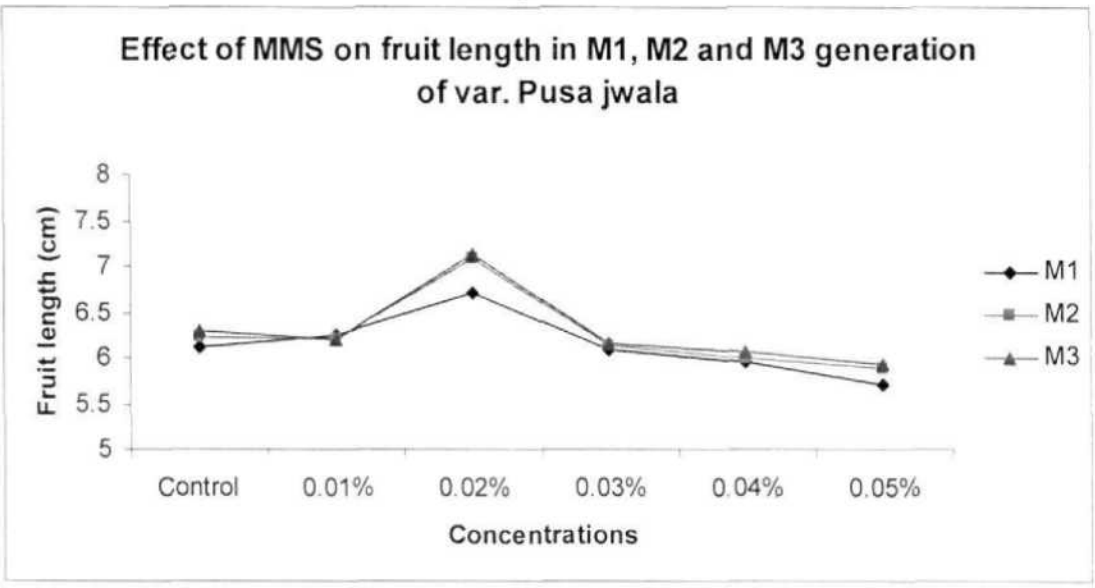
Graph – 24



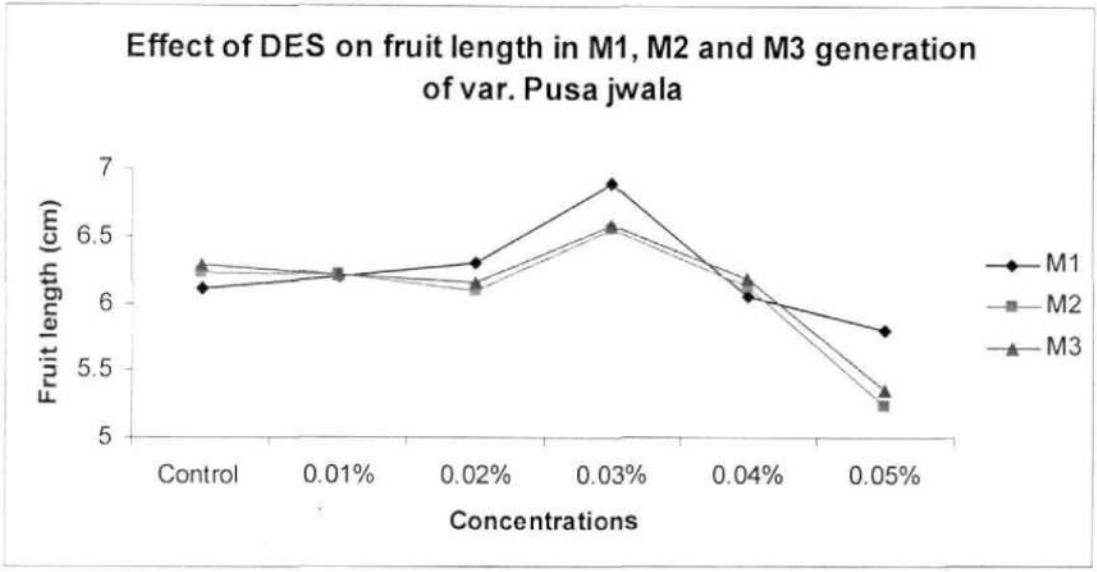
Graph – 25



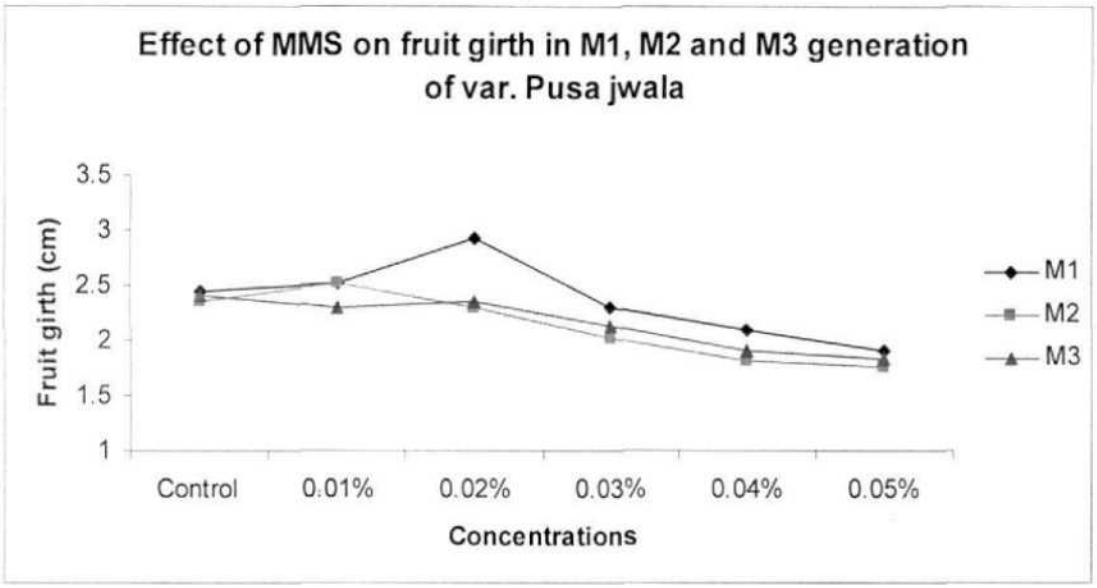
Graph – 26



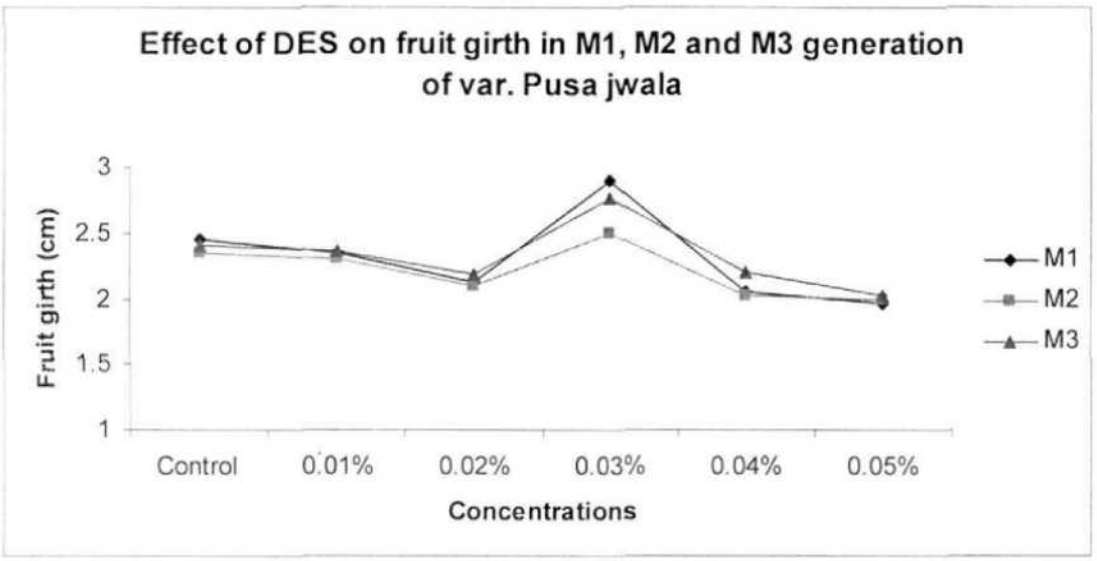
Graph – 27



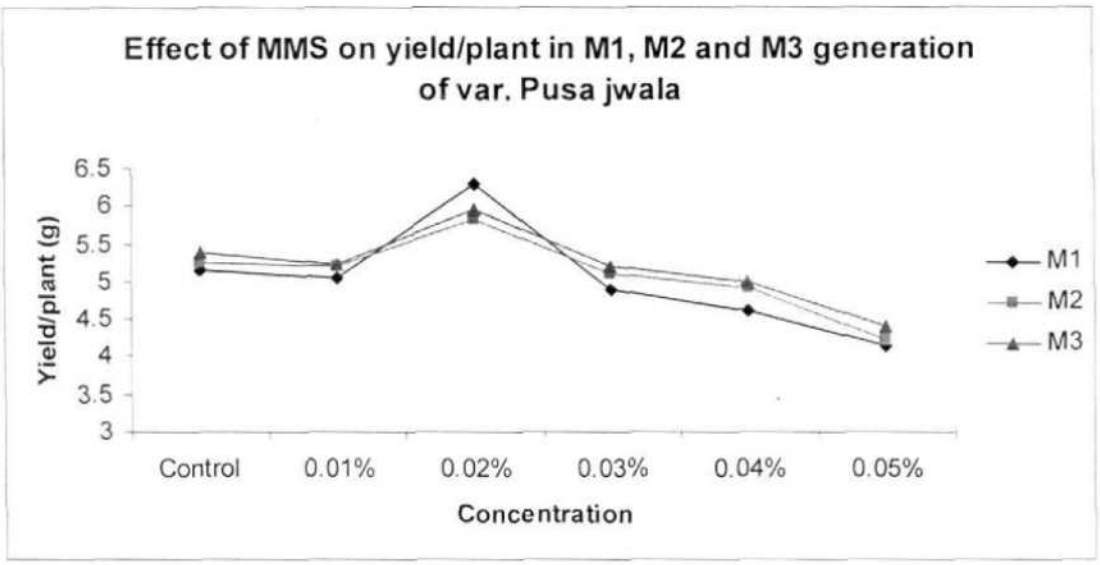
Graph – 28



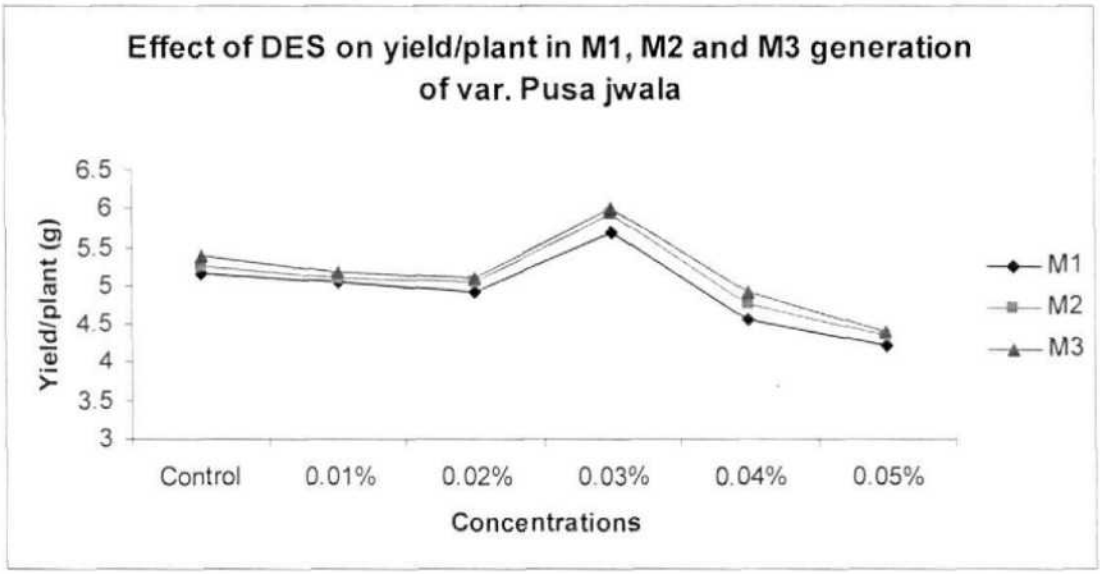
Graph – 29



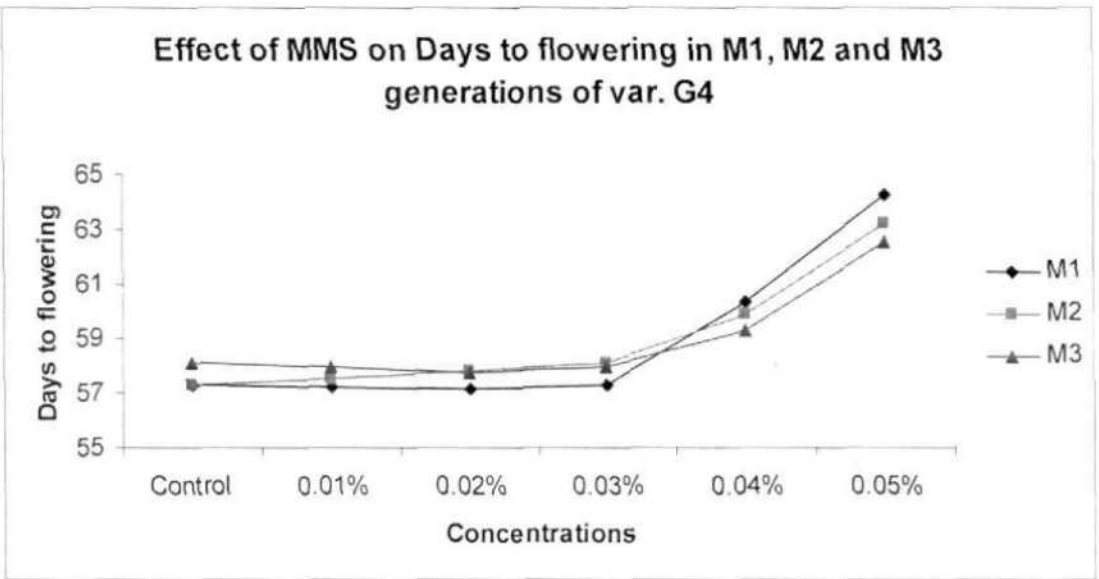
Graph – 30



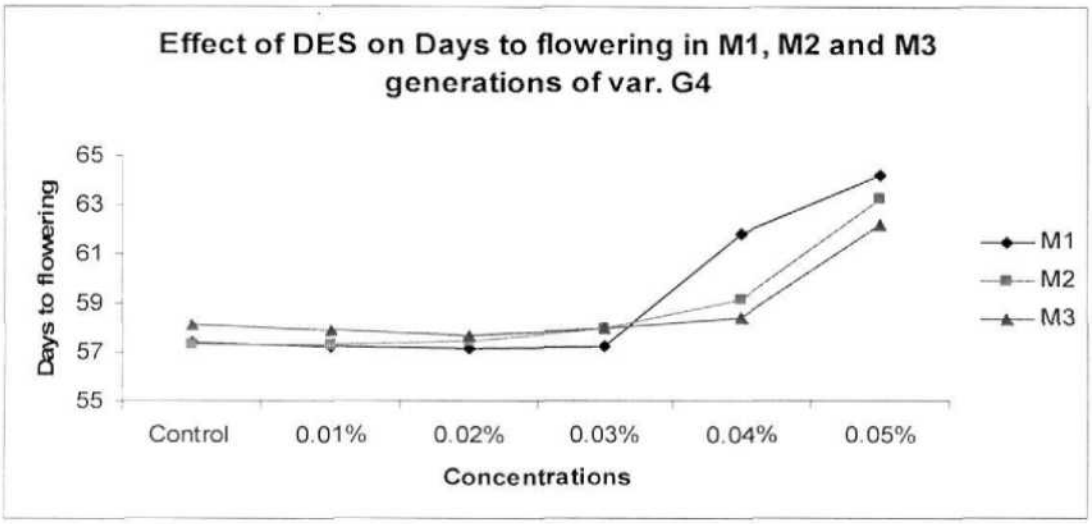
Graph – 31



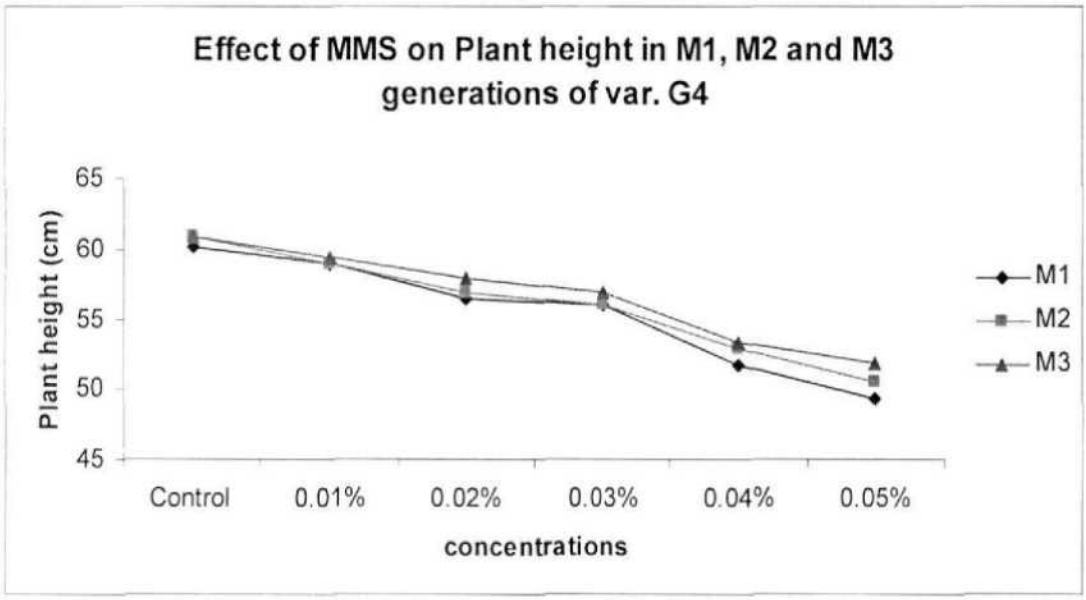
Graph – 32



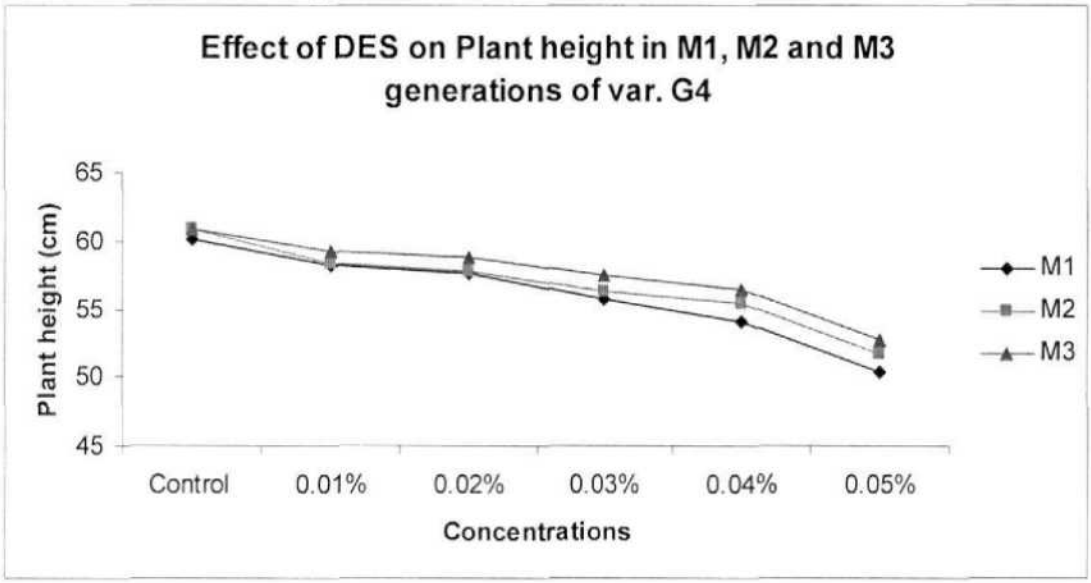
Graph – 33



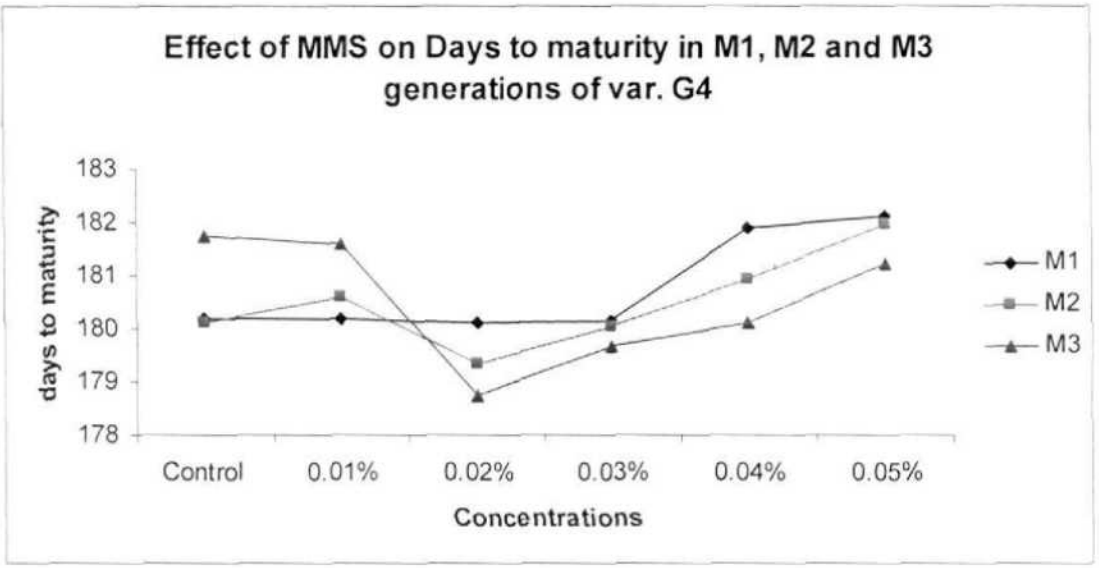
Graph – 34



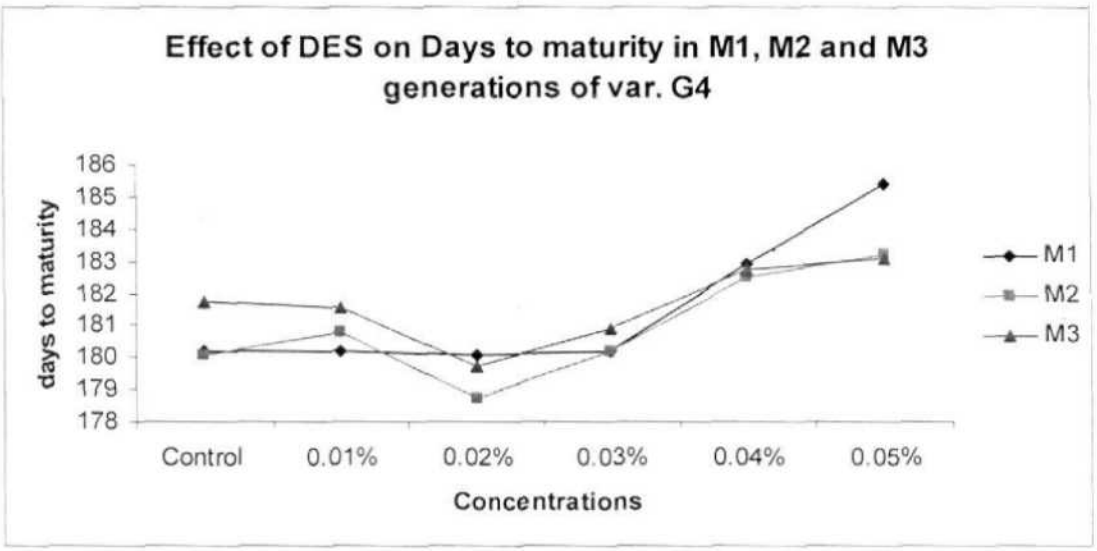
Graph – 35



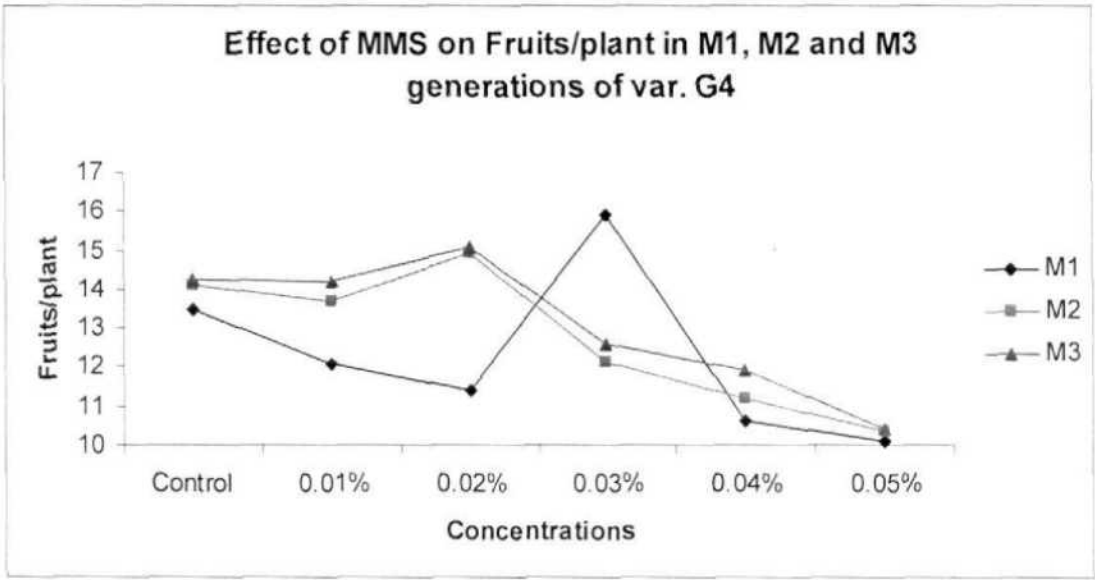
Graph – 36



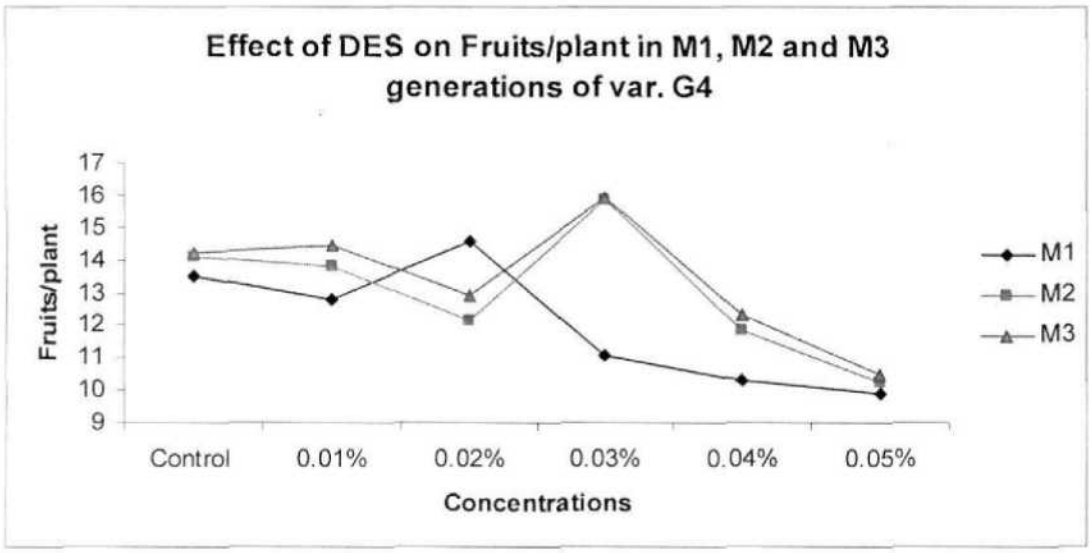
Graph – 37



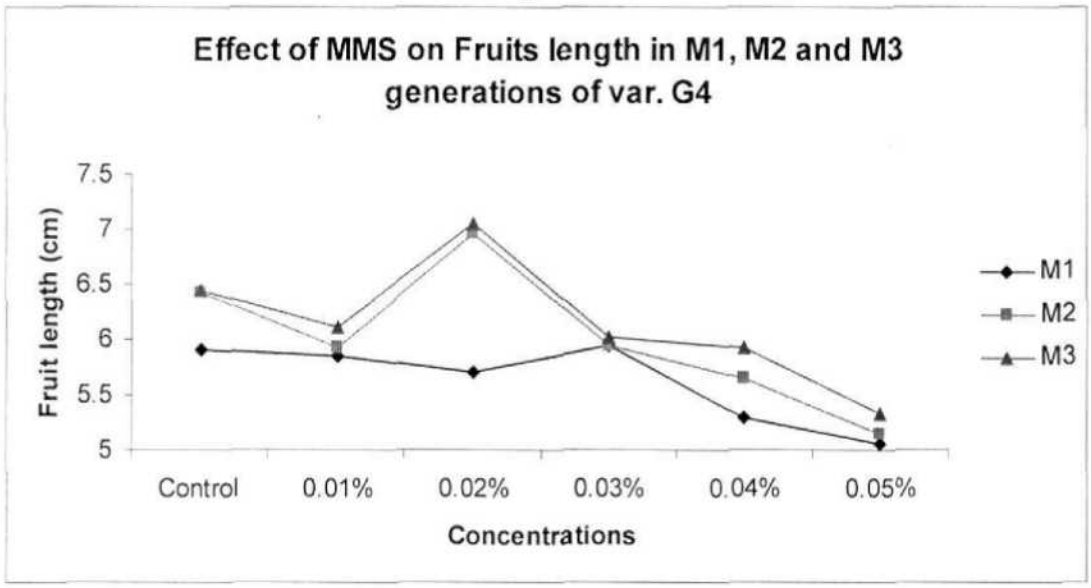
Graph – 38



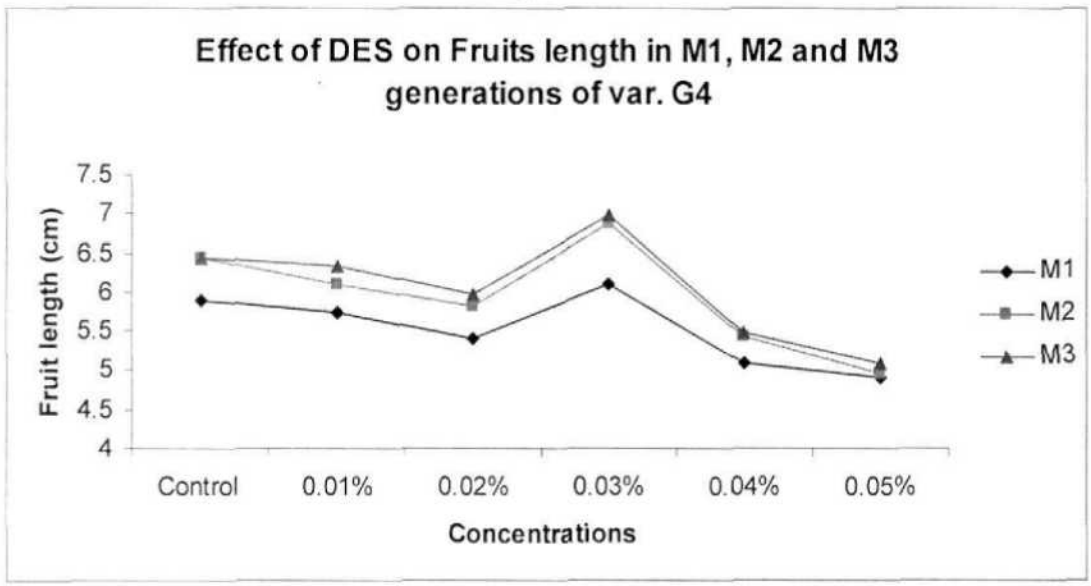
Graph – 39



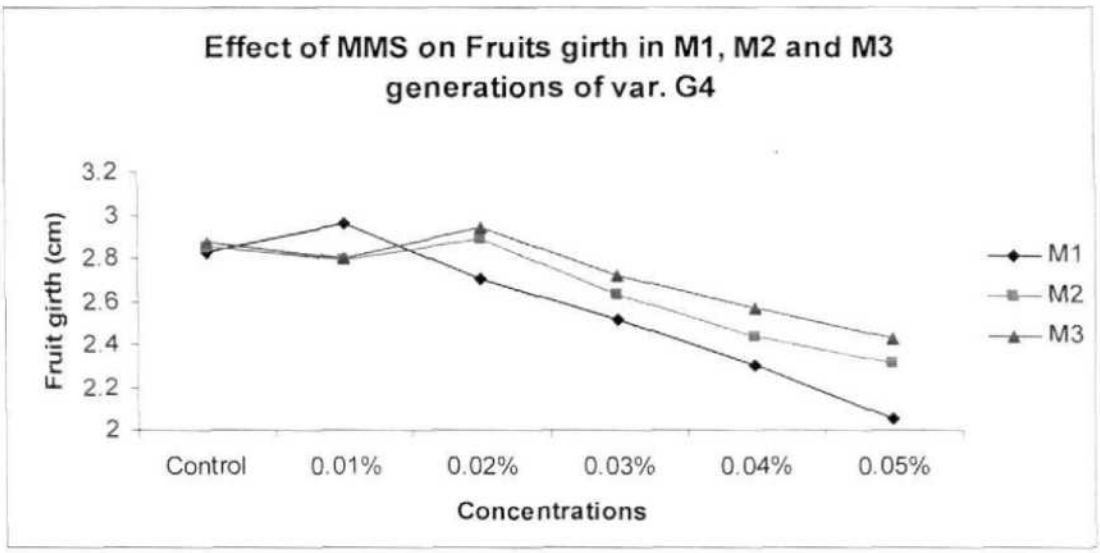
Graph – 40



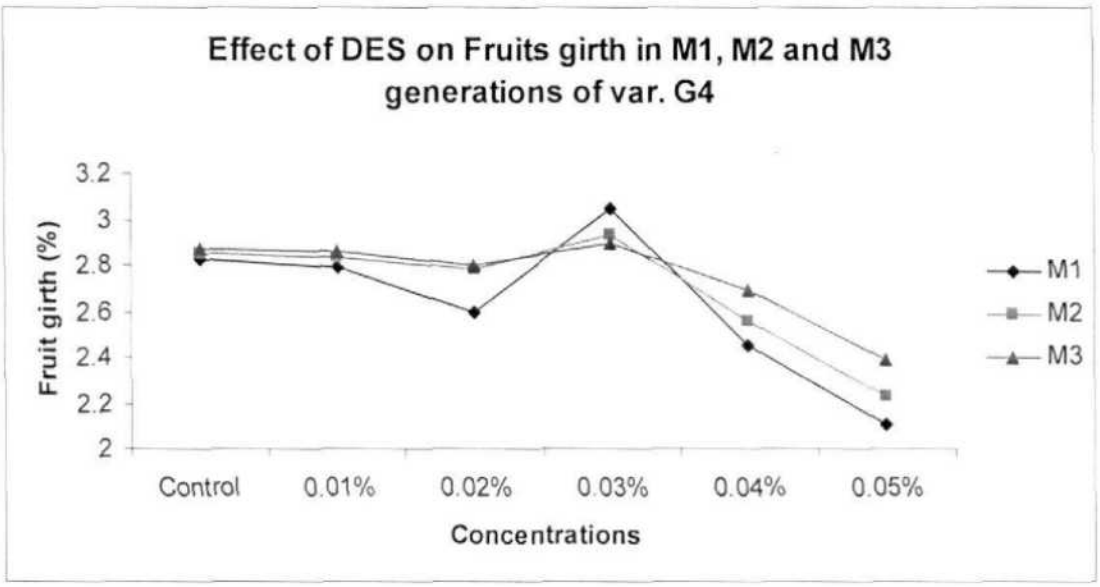
Graph – 41



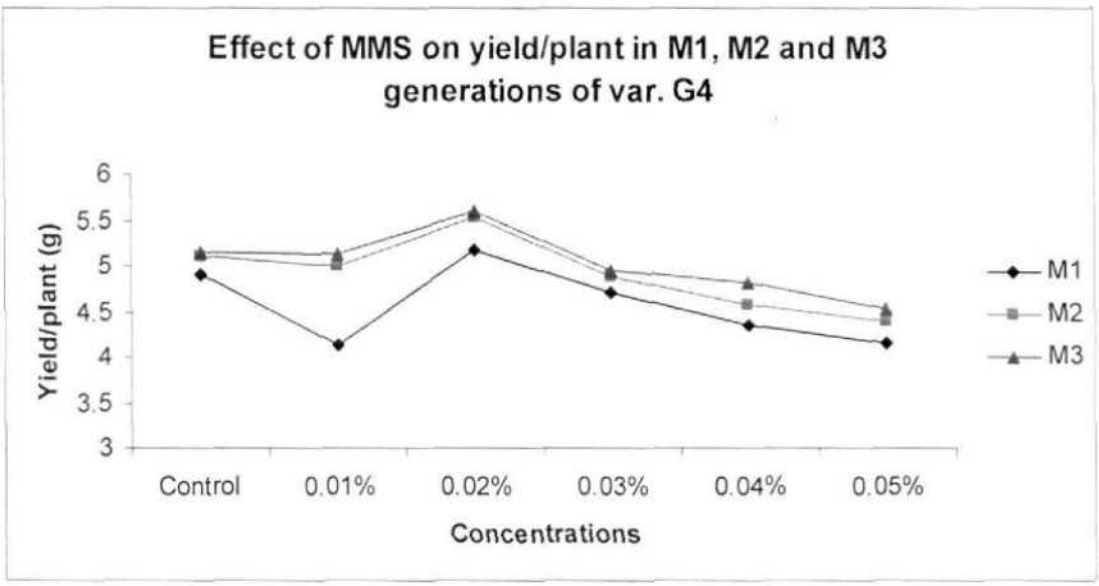
Graph – 42



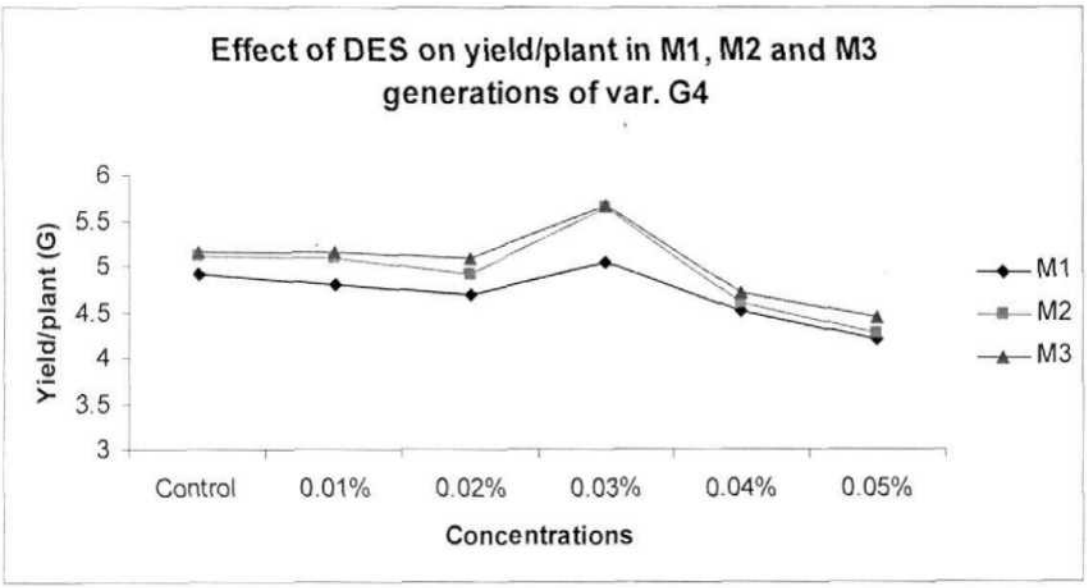
Graph – 43



Graph – 44



Graph – 45


Effect of DES on yield/plant in M1, M2 and M3 generations of var. G4
Yield/plant (G)
6
5.5
5
4.5
4
3.5
3
Control
0.01%
0.02%
0.03%
0.04%
0.05%
Concentrations
M1
M2
M3

Graph – 46

Representative Photographs of Meiotic Abnormalities.

Plate-I

Figs. 1 & 2. Twelve bivalents at diakinesis (control).

Figs. 3 & 4. Twelve 12 bivalents at metaphase-I (control).

Fig. 5. Equal separation (12:12) of chromosomes at anaphase-I (control).

Fig. 6. Two nuclei telophase-I (control).

Fig.7. Metaphase-II (control).

Fig.8. Four groups of chromosomes at anaphase-II (control).

Fig.9. Four nuclei at telophase-II (control).

Figs. 10 &11. Sticky chromosomes at metaphase-I.

Fig. 12. Stray bivalent at metaphase-I.

Plate - I

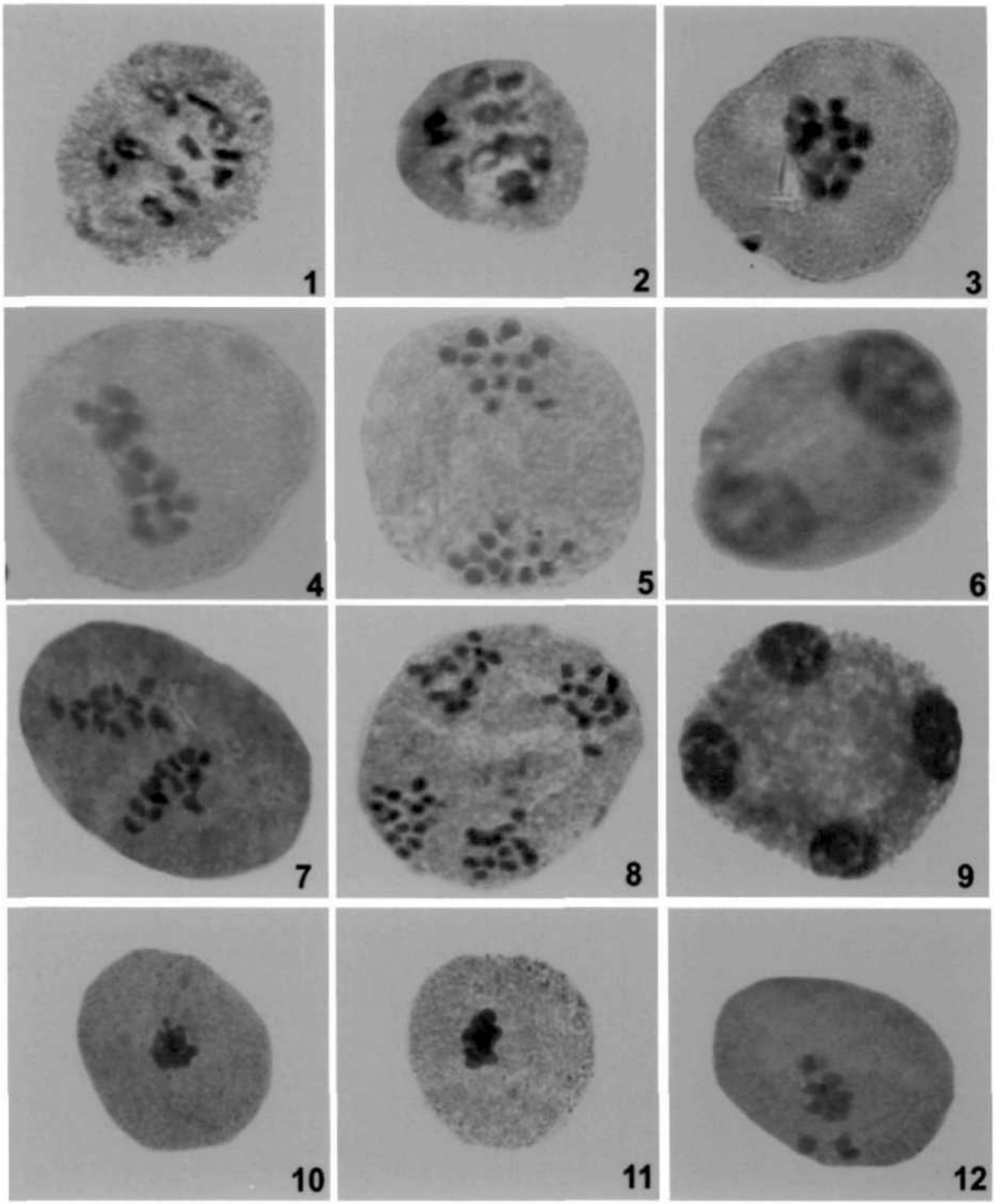


Plate-II

Figs. 1 & 2. Stray bivalents at metaphase-I

Fig. 3. Precocious separation of chromosome at metaphase.

Figs. 4 & 5. Non synchronization of chromosome at metaphase-I & II.

Figs. 6 and 7. Showing univalents at disturbed metaphase-I.

Fig. 8. Univalents, bivalents and multivalent at metaphase-I.

Fig. 9. Univalents at late diakinesis.

Fig. 10 showing many univalents at metaphase-I.

Figs. 11 & 12. Non synchronous movement of chromosomes at anaphase-I.

Plate - II

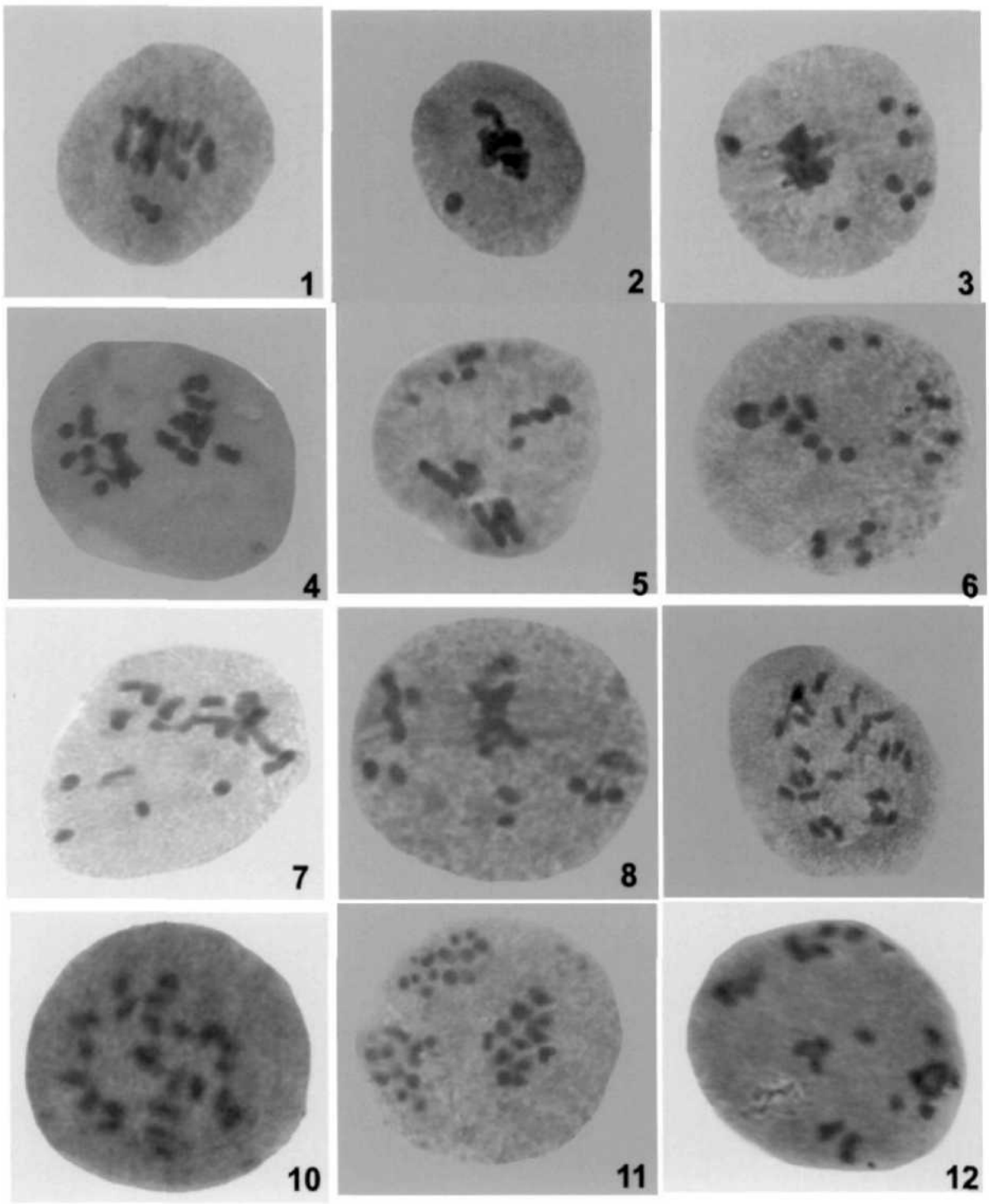


Plate-III

Figs.1 & 2. Stray bivalents at metaphase-I and II.

Fig. 3. showing unequal separation of chromosome (11:13) at anaphase-I.

Fig. 4. Laggards at anaphase-II

Figs. 5, 6 & 7 Showing laggards at anaphase-I

Fig. 8. Chromatin bridges at anaphase-I

Fig. 9. Unequal separation of chromosomes (9:15) at anaphase-I

Fig. 10, 11 & 12. Laggards at anaphase-I

Plate - III

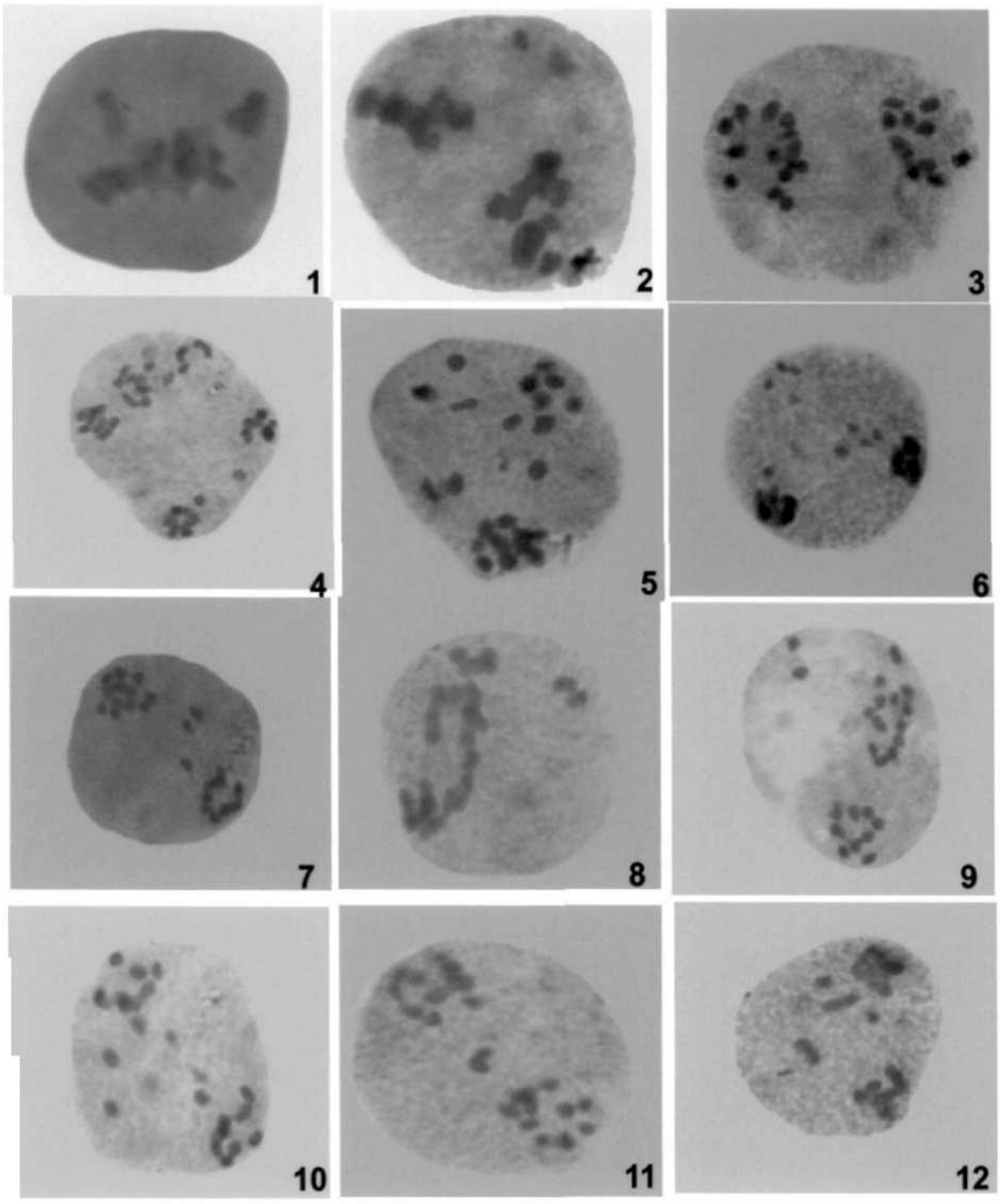


Plate-IV

Fig. 1. Laggards at anaphase-I

Fig. 2. Chromatin bridge and univalents at anaphase.

Fig. 3. One chromatin bridge and one univalents at anaphase-I

Fig. 4. Disturbed polarity at telophase-II.

Fig. 5 & 6. Disturbed polarity and micronuclei at telophase-II.

Fig. 7. Disturbed polarity at telophase-II.

Fig. 8. Micronuclei at telophase-I.

Fig. 9. Bridge with fragment at telophase-II.

Fig. 10. Chromatin bridge at telophase-II.

Fig. 11. Brocken bridge at telophase-II.

Fig. 12. Six nuclei and one micronucleus at telophase-II.

Plate - IV

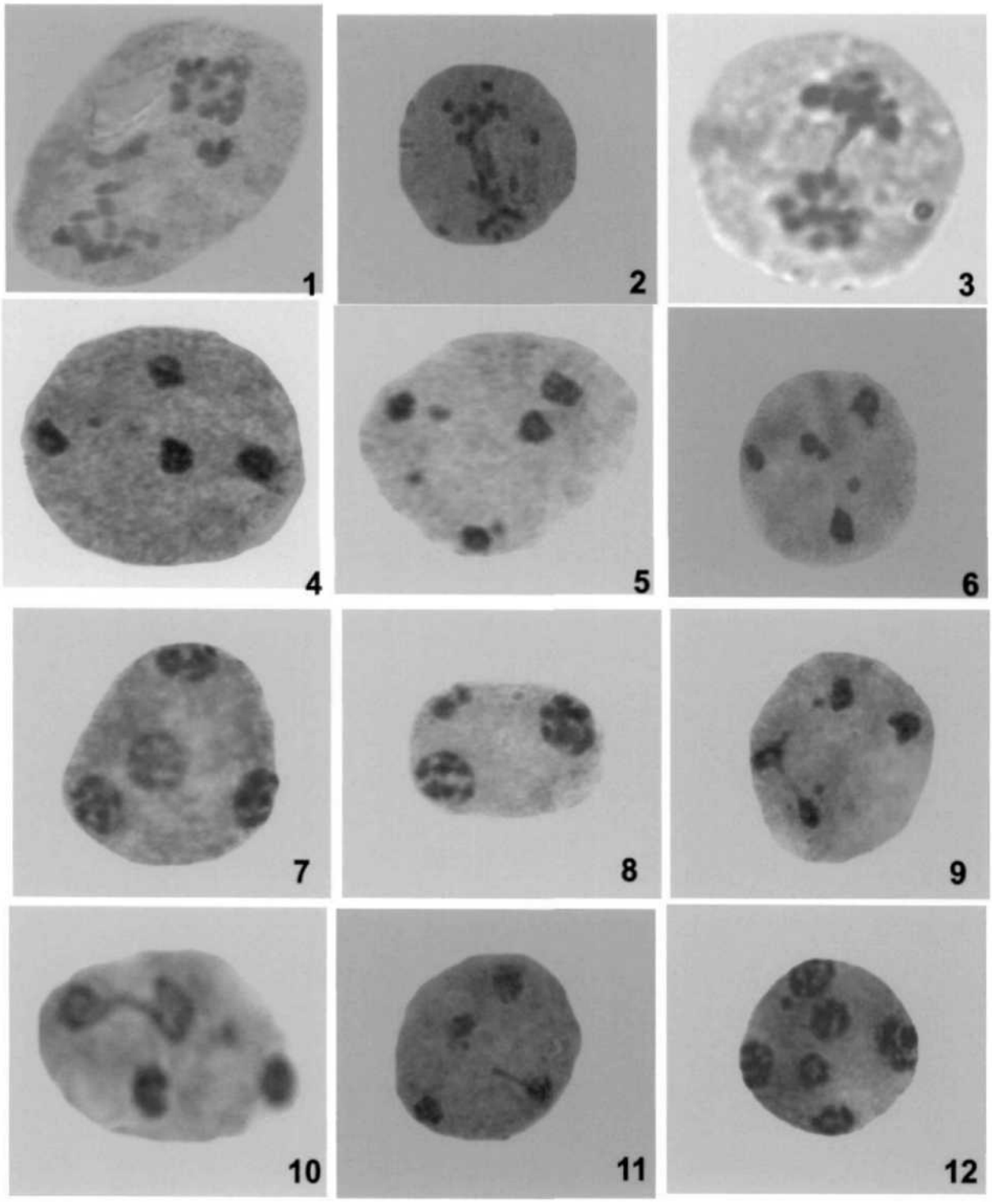
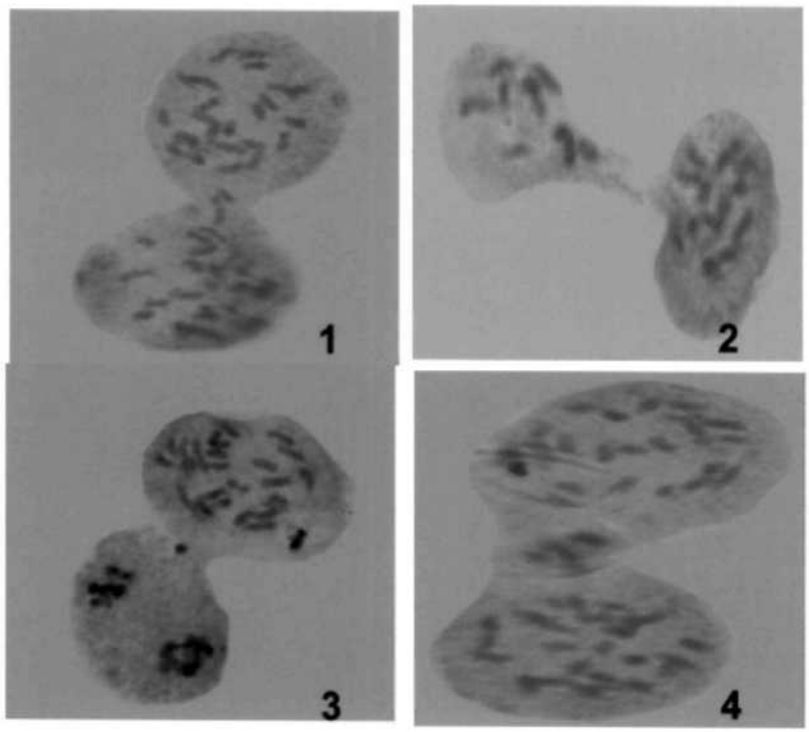


Plate-V

Figs. 1, 2, 3 and 4 showing cytomixis at various stages of meiosis.

Plate - V



Selected mutants in M_3 generation of var. Pusa jwala

Plate- VI

Figs. a & b. Normal (control) plants of var. Pusa jwala.

Plate - VI

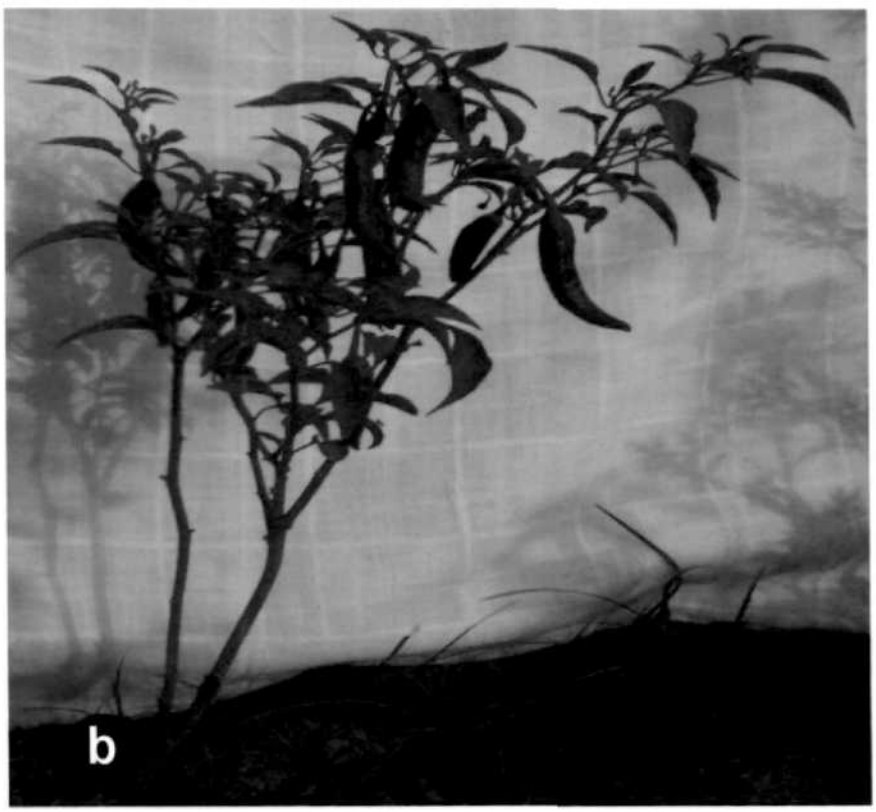


Plate- VII

Fig. a. Highly branched and heavily fruited mutant plant

Fig.b. Close view of fig. a.

Plate - VII

Plate-VIII

Fig. a. Tall mutant with increased number of fruits.

Fig.b. Close view of fig.1

Plate - VIII

Plate-IX

Fig. a. Mutant with long and thick fruits.

Fig. b. Red colored fruits at maturity.

Fig.c. Close view of fig.b

Plate - IX

Plate-X

Fig. a. Mutant showing short thick fruits.

Fig.b. Close view of fig. a.

Fig.c .Mutant showing red coloured fruits at maturity.

Plate - X

Plate-XI

Fig.a. Mutant with large number of fruits at maturity.

Fig.b. Close view of fig. a.

Plate - XI

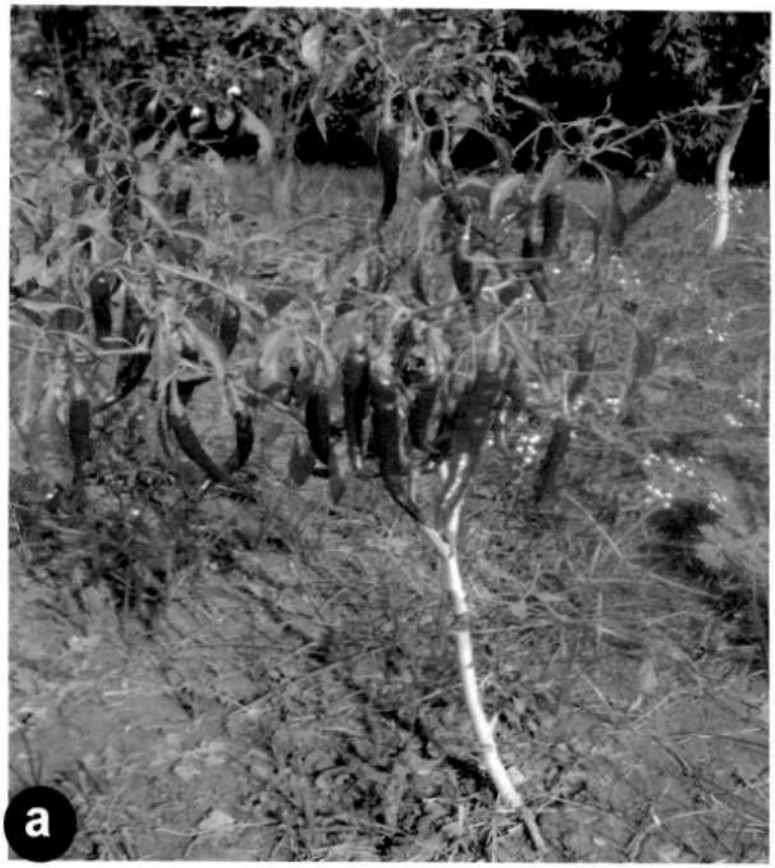


Plate-XII

Early maturing mutant.

Plate - XII

Selected mutants in M_3 generation of var. G4.

Plate-XIII

Fig. a. Normal (control) fruited plant.

Plate - XIII

Plate-XIV

Fig. a. Dwarf mutant with long fruits.

Fig. b. Close view of fig.a.

Plate - XIV

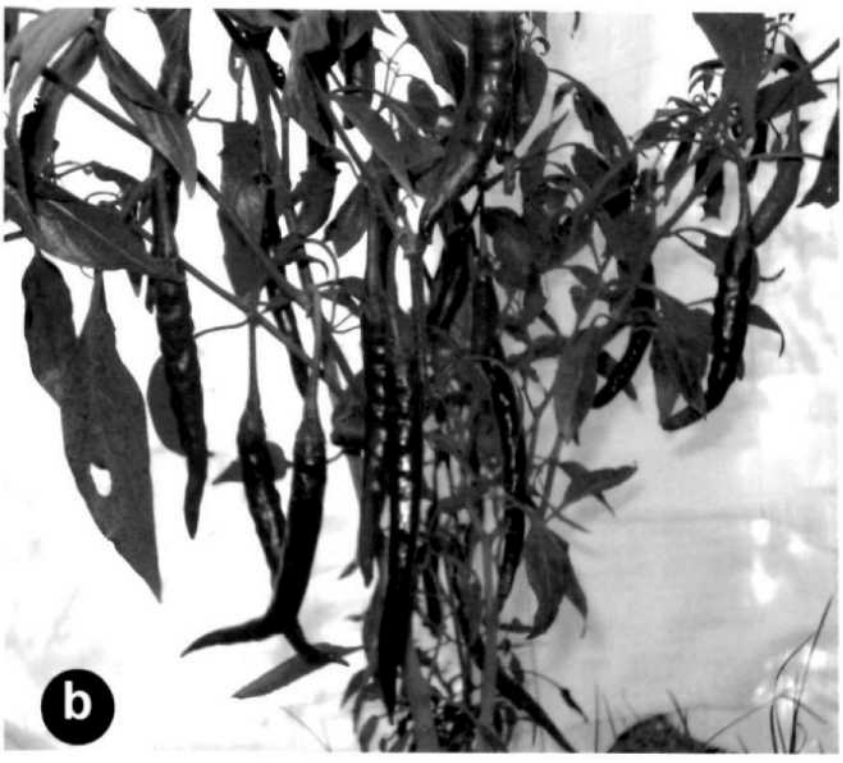


Plate-XV

Fig. a. Highly branched mutant with short fruit.

Fig. b. Mutant showing short red fruits at maturity.

Plate - XV

Plate-XVI

Fig. a. Tall mutant with large number of thin, long and red fruit.

Fig. b. Close view of fig. a.

Plate - XVI

Plate-XVII

Fig. a. Early maturing mutant with long fruits.

Plate - XVII

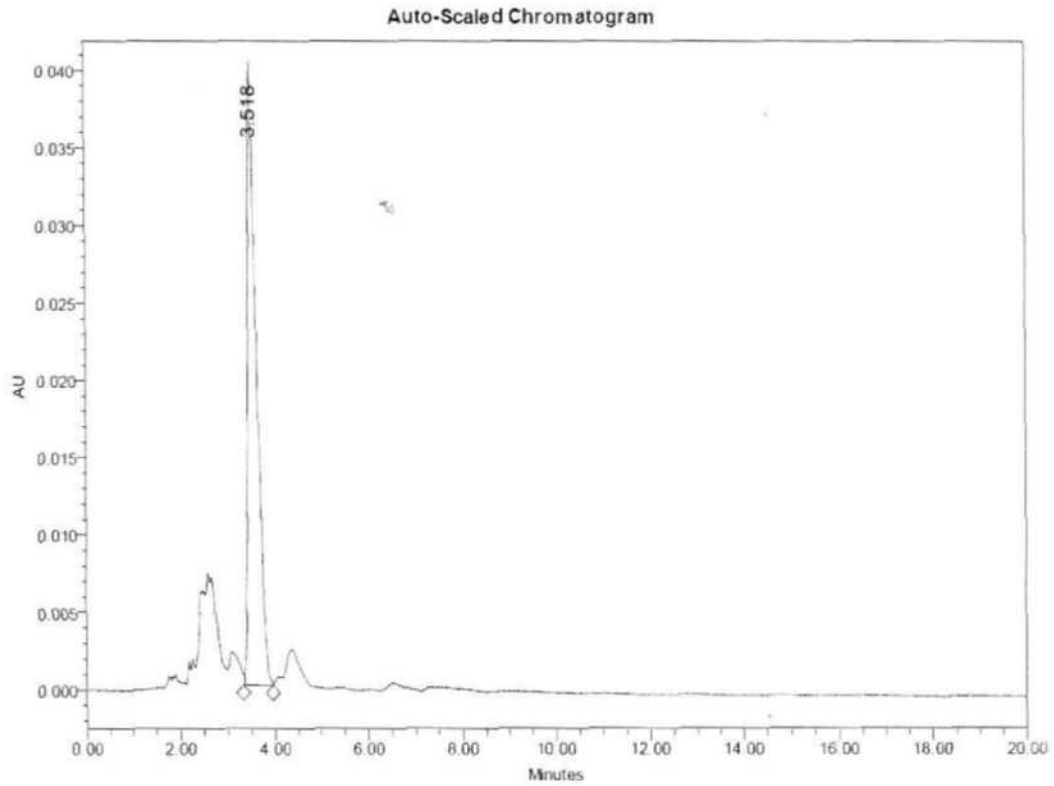


Fig. 1: HPLC Chromatogram of standard Capsaicin Solution

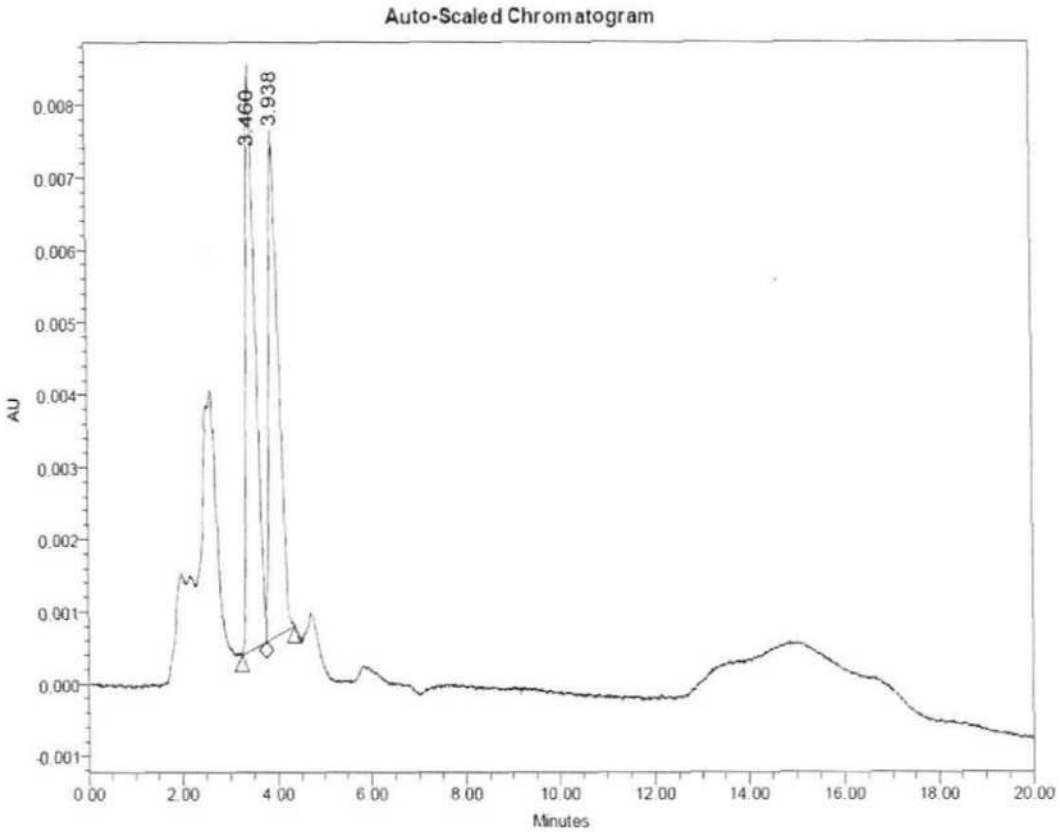


Fig. 2: HPLC Chromatogram of Control Plant of Var. Pusa Jwala

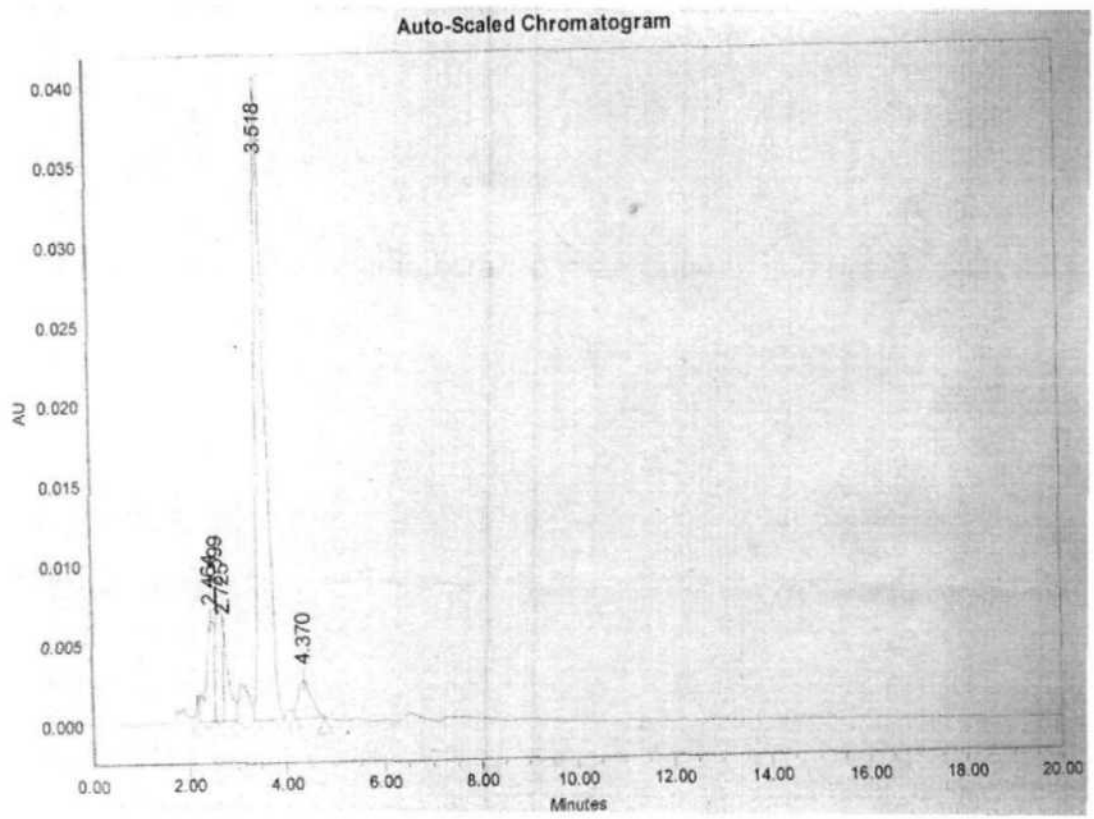


Fig. 3: HPLC Chromatogram of Selection-I of Var. Pusa Jwala

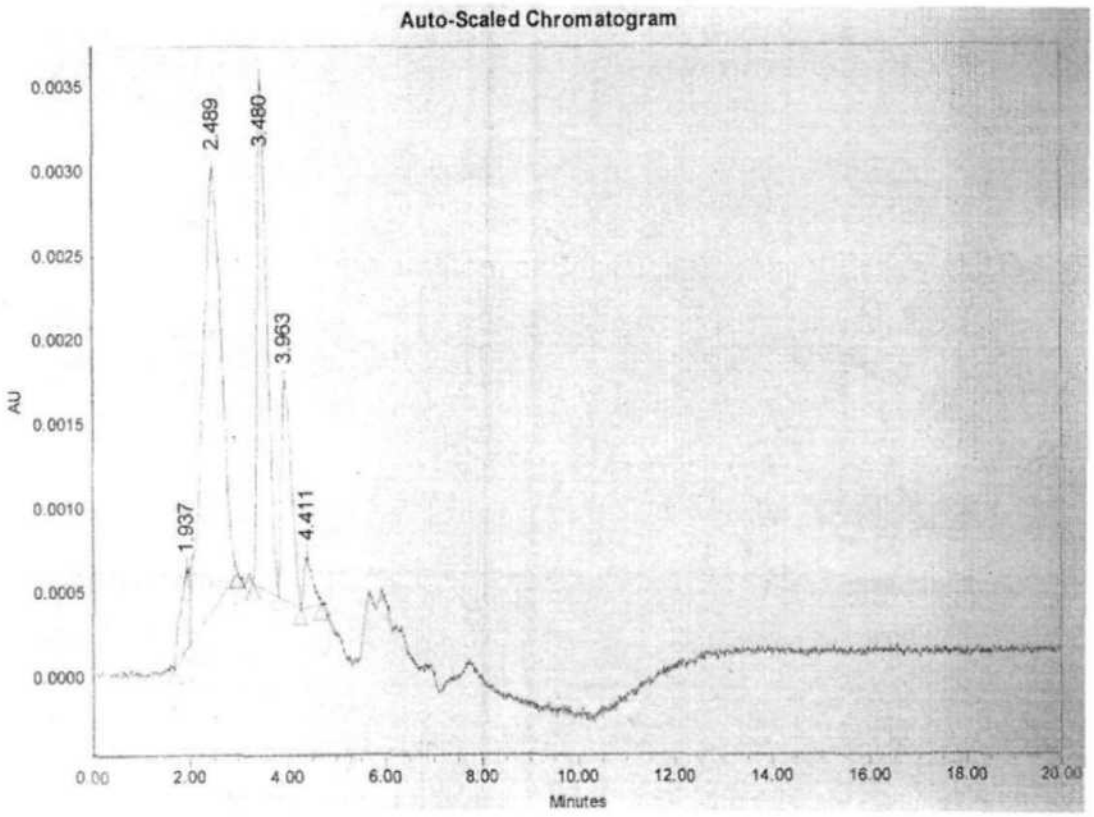


Fig. 4: HPLC Chromatogram of Selection-II of Var. Pusa Jwala

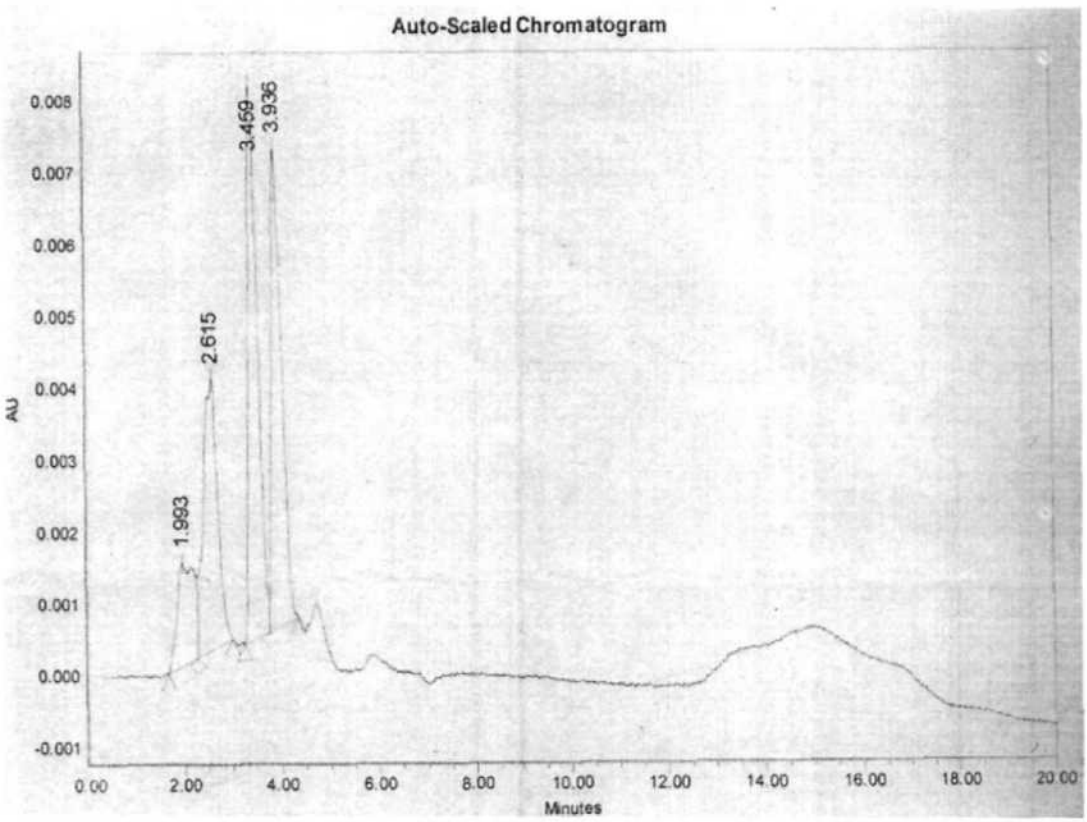


Fig. 5: HPLC Chromatogram of Selection-III of Var. Pusa Jwala

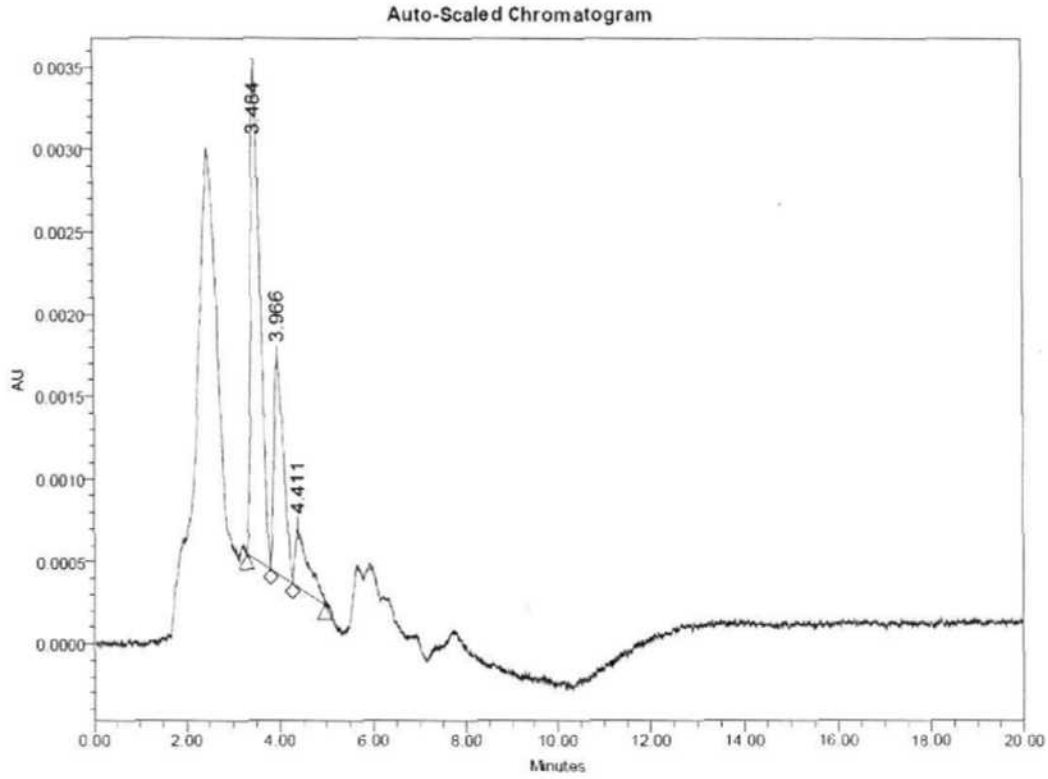


Fig. 6: HPLC Chromatogram of Selection-IV of Var. Pusa Jwala

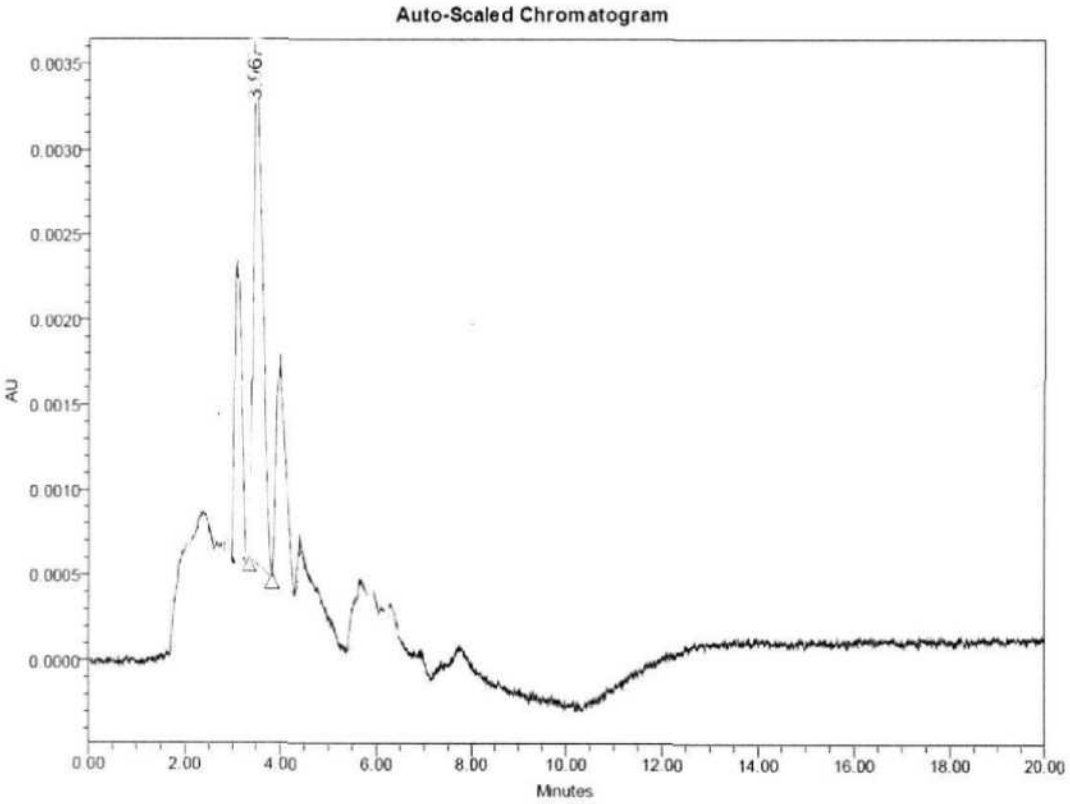


Fig. 7: HPLC Chromatogram of Selection-V of Var. Pusa Jwala

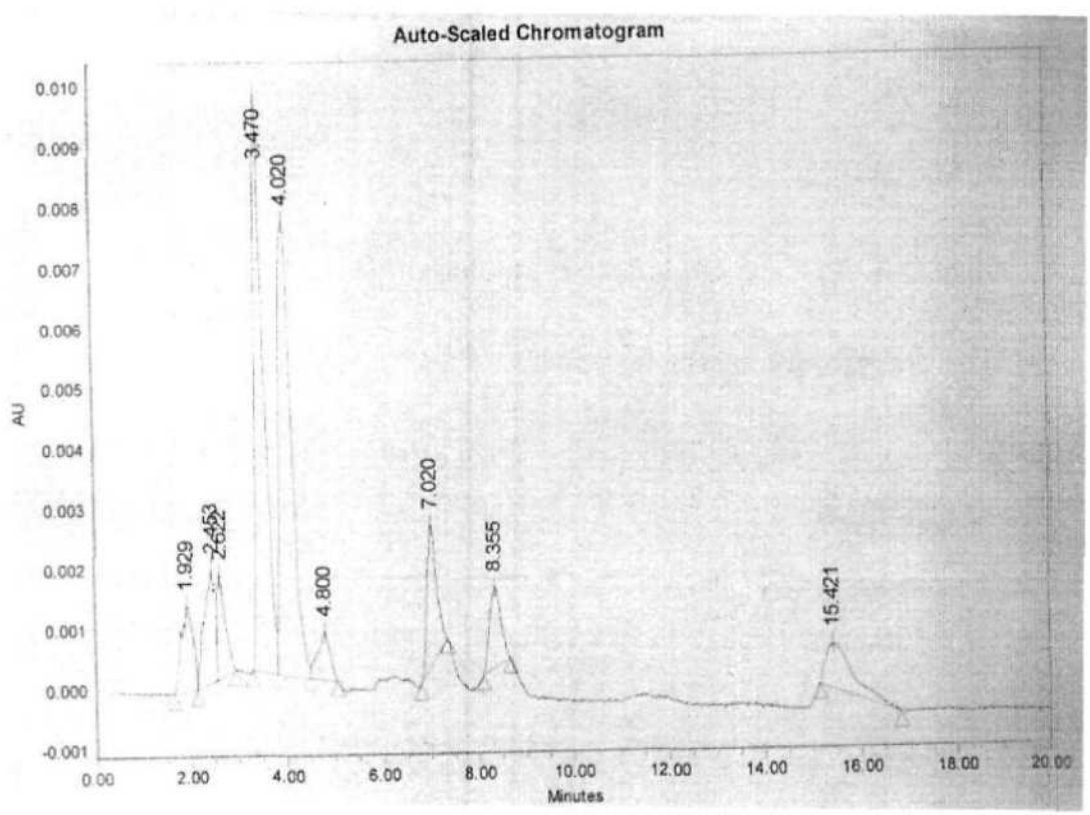


Fig. 8: HPLC Chromatogram of Control Plant of Var. G4

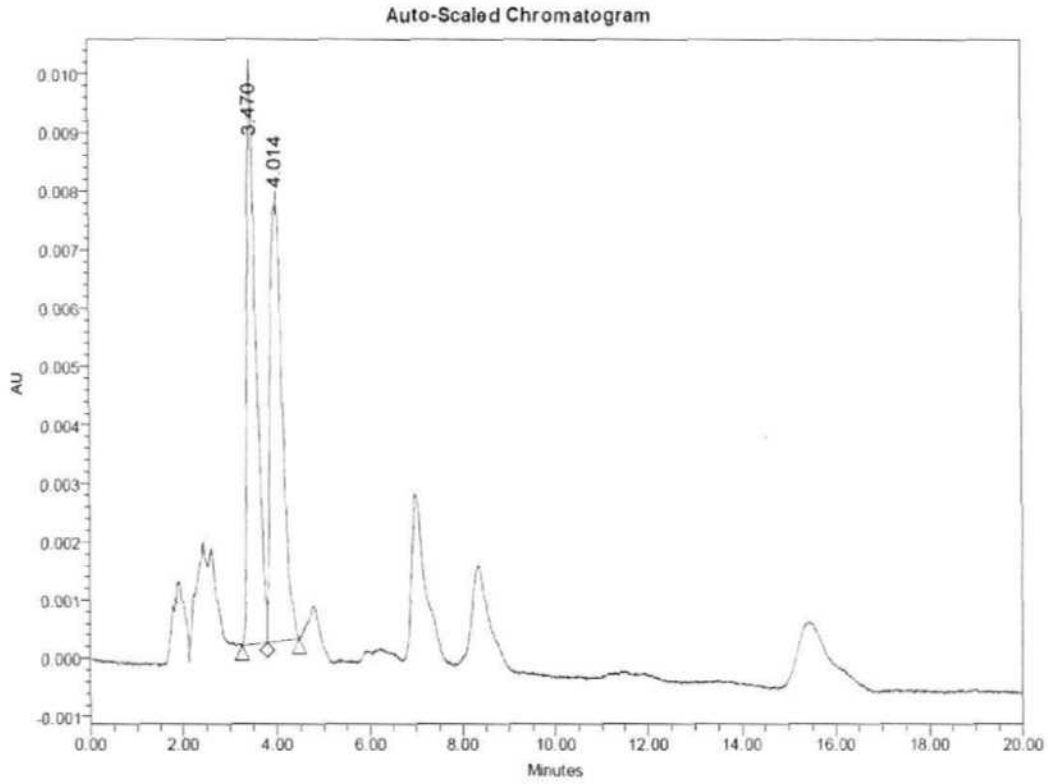


Fig. 9: HPLC Chromatogram of Selection-I of Var. G4

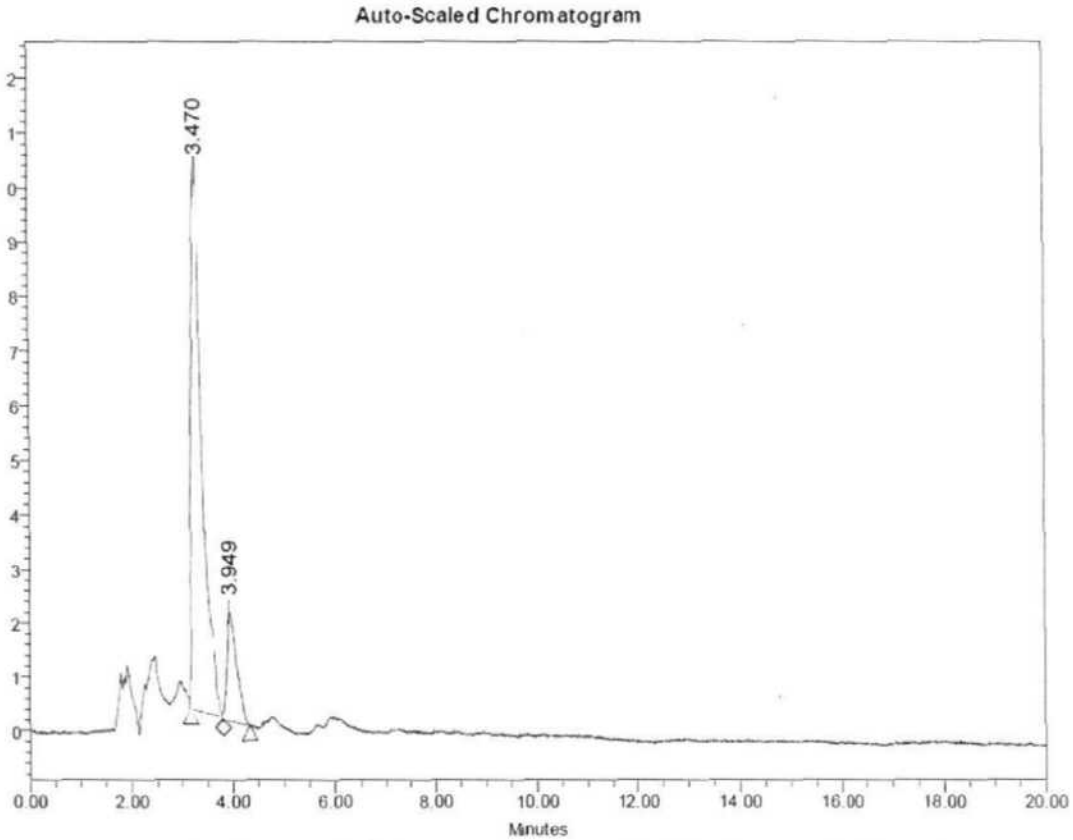


Fig. 10: HPLC Chromatogram of Selection-II of Var. G4

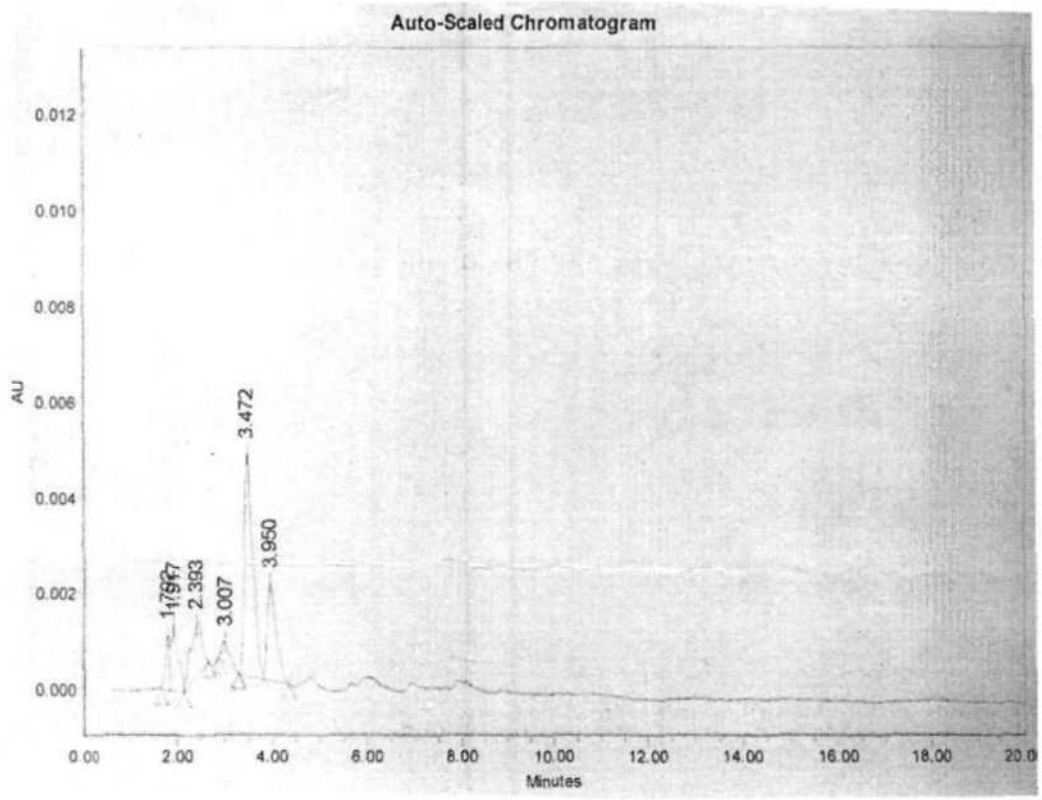


Fig. 11: HPLC Chromatogram of Selection-III of Var. G4

Chapter-5

DISCUSSION

DISCUSSION

The breeding potential of a crop plant is to exploit the existing genetic variabilities through selection or induced variabilities. Mutation breeding technique is one of the best methods to enlarge the genetically conditioned variabilities in a species within a short period of time and has played a significant role in the development of several new crop varieties (Micke, 1988). Induced mutagenesis plays very important role in enhancing genetic variabilities for crop improvement by inducing micromutations in addition to the visible macromutations and is the simplest and fastest way to isolate mutants of agronomic and economic significance. The primary strategy in mutation based plant breeding has been to upgrade the well adopted varieties by altering one or two major traits. These include mainly the yield contributing traits. Besides, induced mutagenesis offers a possibility for the induction of desirable attributes perhaps those that either can not found naturally or have been lost.

The induced mutagenesis finds a prominent place in the augmentation and re-creation of genetic variability which was lost by a rigid selection or narrow base of germplasm of a crop plant under improvement. The potentiality of mutations for this purpose however, depends upon the efficiency of induction of mutation (Siddqui and Yousufzai, 1988). The enhancement of mutation frequency and the alteration of mutation spectrum in a predictable manner remain the all times important aspects of mutation research. Increased number of mutated genes over a certain threshold essentially needs an extensive research for refined methods and treatment condition. It is often suggested that the manipulation of sieves in the mutation process would seem one means of obtaining a certain degree of phenotypic specificity (Siddiqui and Jafri, 1986). To ensure a speedy generation of variability for a specific trait to be improved, a mutation breeder has to go through all basic events met in the methodology to ensure reliable information about the mutagenic sensitivity of biological material and the extent of effectiveness and efficiency of mutagen in question. Mutagens vary in their mode of action, effectiveness, efficiency and the spectrum of mutations induced. Similarly, genotypes show differential sensitivity towards mutagens even at varietal level.

The basic information on mutagenic sensitivity, efficiency of mutagens, methods of handling the material and treatment methods required to maximize mutation induction is essential for any mutation breeding programme. Numerous mutant varieties through induced mutation have developed significant economic impact, sustaining crop production and greatly contributing to increase of food production. According to IAEA mutant varieties data base (2009), 3100 new crop varieties, all carrying novel induced variations have been officially registered.

The present investigation was planned to estimate mutagenic effects of Methyl methane sulphonate (MMS) and Diethyl sulphate (DES) on cytomorphological characters of *Capsicum annuum* L. var. Pusa jwala and G_4 in M_1, M_2 and M_3 generation. The results obtained during the course of present investigation have been discussed in this chapter.

5.1 MORPHOLOGICAL STUDIES:

5.1.1 Seed germination:

Seed germination is an important parameter to estimate the effect of mutagens on plants. The reduced seed germination due to inhibitory effects of mutagens has been reported earlier by several workers (Alcantara *et al.*, 1996; Rangaiah *et al.*, 2002; Jabeen and Mirza, 2002; Raghuvanshi and Singh, 2007). Similar inhibitory effect of mutagens on seed germination in *Lathyrus sativus* L. (Kumar & Dubey, 1998a), *Cicer arietinum* (Jabee & Ansari, 2005), *Vicia faba* (Agarwal & Ansari, 2001; Khan *et al.*, 2006a; Bhat *et al.*, 2007a), *Zea mays* (Kumar & Rai, 2007a), *Trigonella foenum-graecum* (Siddiqui *et al.*, 2007; Jabee *et al.*, 2008), *Helianthus annuus* (Khursheed *et al.*, 2008), *Cichorium intybus* (Ahmad *et al.*, 2009), has also been demonstrated. The similar inhibitory effect of MMS and DES on seed germination has also been observed in present investigation on *Capsicum annuum* L.

Several workers have attempted to explain the causes responsible for inhibition in seed germination. It may be due to inhibition of growth regulators (Sideris *et al.*, 1971) and metabolic disturbances during germination (Ananthaswamy *et al.*, 1971). Griffith and Johnson (1962) and Srivastava (1979) considered that reduction in germination percentage was due to weakening and disturbances of growth process regulated in early stages of germination. Krishna *et al.* (1984)

considered that the mutagen reached inside the seed through imbibition process and imbalanced the cell system, inhibiting the normal metabolic activity. Kumar and Rai (2007b) have reported that reduction in seed germination is due to the effect of mutagen on meristematic tissues of the seed and due to the chromosomal damage caused by mutagen. It may also be due to the presence of incompletely developed embryos in seeds (Falque, 1994). The decrease in the percentage of seed germination after mutagenic treatment may be ascribed to the chromosomal aberrations, disturbances in DNA and auxin synthesis and to the impaired cell metabolism (Kirtane and Dhumal, 2004).

5.1.2 Plant Survival:

Reduction in plant survival with increasing concentrations of mutagens has observed in the present investigation in both the varieties of *Capsicum annuum* L., has also been reported by Vandana and Dubey (1988) in faba bean, Edwin and Reddy (1993) in triticale, Kumar and Dubey (1998a) in khesari, Dhamayanthi and Reddy (2000) in chilli, Kalia *et al.*, (2001) in wheat, Khursheed *et al.*, (2008) in sunflower and Aslam *et al.*, (2012) in *Cichorium intybus* L. The reduction in survival at higher mutagenic level has been attributed to various factors, such as the chromosomal damage leading to mitotic arrest (Khursheed *et al.*, 2008), changes in the metabolic activity of the cells (Natarajan and Shivashankar, 1965), inhibitory effect of mutagen (Sree Ramulu, 1972) and balance between growth promoters and inhibitors (Meherchandani, 1975). Physiological imbalance or different types of chromosomal aberrations or both may be the main cause for drastic decrease in survival (Rao, 1983). Decrease in plant survival may be attributed to the series of events at the cellular level which affect the vital macromolecules and bring about a physiological imbalance in the cells or chromosomal damage leading to mitotic arrest as a consequence of exposure to mutagens.

5.1.3 Pollen fertility/sterility:

The reduction in pollen fertility was found to be dose dependent in both the varieties and decreased with increasing concentrations of both the mutagens. It was comparatively lower in the first generation in all mutagenic treatments but gradually increased in the subsequent generations. The negative effect of mutagens on pollen

fertility may be due to cumulative effects of various meiotic aberrations (Jabee & Ansari, 2005; Khan *et al.*, 2009a). Chromosomal anomalies like univalents, multivalents, stickiness, laggards, bridges, micronuclei etc. are closely associated with pollen sterility in mutagen treated populations (Reddy & Rao, 1981, 1982; Singh, 1992; Anis & Wani, 1997; Kumar & Tripathi, 2004; Kumar & Rai, 2007c; Cali, 2008; Jabee *et al.*, 2008) and the accumulation of more and more chromosomal abnormalities greatly affected microsporogenesis leading to the formation of non-viable gametes, which considerably reduced plant fertility (Kumar & Rai, 2007c & Cali, 2009). Ramesh and Reddi (2002) suggested that mutagen induced pollen sterility could be chromosomal, genic or physiological in nature, while Sharma *et al.*(2004) attributed it mainly due to chromosomal aberrations. The fact that meiotic abnormalities are responsible for pollen sterility has been supported by Sinha and Godward (1972), Kaul (1990), Pagliarini and Pereira (1992), Zeerak (1992), Pagliarini *et al.* (1993), Consolaro *et al.* (1996), Taschetto and Pagliarini (2004), Khan *et al.* (2009a) etc. According to Reddi (1977) the pollen sterility was the result of interchange of segments between non-homologous chromosomes. Low chiasma frequency may be one of the causes of low pollen fertility, because chiasmata are responsible for the maintenance of the bivalents which permit normal chromosome segregation and this process ensures pollen fertility (Defani Scoarize *et al.*, 1995a; Pagliarini, 1990; Consolaro *et al.*, 1996). Srivastava and Kapoor (2008) reported that spindle related aberrations like tripolarity, multipolarity and unorientation may cause the formation of unbalanced and sterile gametes affecting the plant fertility. It is suggested that failure of homologous pairing during meiosis could be the reason of high pollen sterility.

Studies on germination, survival and fertility/sterility revealed that much of the inhibitory effects were recovered in the second generation, although the higher concentration treatments of both the mutagens still retained the adverse effects. Such a recovery mechanism in M_2 generation has also been reported by different workers (Katiyar, 1978a; Jayabalan and Rao, 1987a; Subba Rao, 1988).

5.1.4 Seedling and plant height:

In the present investigation the average height of seedlings and plants decreased with increasing concentrations of mutagens used. Similar results were observed in the same plant by several workers (Jabeen and Mirza (2002); Sharma and Anis (1995) and Omar *et al.* (2008). Reduction in seedling and plant height was also observed by Vandana & Dubey (1988) and Agarwal and Ansari (2001) in *Vicia*, Singh *et al.* (1993) in *Brassica*, Khan (1990) and Das *et al.* (2004) in *Vigna*, Nabipour *et al.*, (2004) in *Helianthus* and Stamo *et al.* (2007) in *Triticum* species. Salam (1990) concluded that the reduction in seedling growth may be due to the gross injury caused at cellular level, either due to gene controlled biochemical process and/or acute chromosomal aberrations. Chromosomal damages or inhibition of cell division may be one of the chief reasons of reduced seedling growth (Gray & Read, 1950; Thoday, 1954; Sparrow *et al.*, 1961; Arumugam *et al.*, 1997). Uneven damage to meristematic cells as a result of genetic injuries and physiological disturbances, caused reduction in seedling and plant growth (Ansari & Siddiqui, 1996). Arumugam *et al.* (1997) considered that the reduction in seedling height after mutagenic treatments is generally due to inhibition of mitotic proliferation and variation in auxin level. It is believed that inhibition of seedling growth may be due to slow rate of cell division, decreased amylase activity and increased peroxidase activities (Rao, 1980; Rao and Rao, 1983).

In present investigation Plant height decreased considerably in the treated populations. Several workers have explained the causes of decreasing height due to mutagenic treatments. According to Gunckel (1957) the possible influence of phytohormones and other physiological disturbances are responsible for stunted plant growth. Gupta and Sumata (1967) reported that auxin had a rapid turn over rate in metabolically active tissues and its biosynthesis is very sensitive to the mutagens, hence affecting the growth. Reduction in the plant height may be due to the chromosomal damage and/or inhibition of cell division (Thoday, 1951; Sparrow *et al.* 1961) whereas, Goud and Nayar (1968) and Tarar and Dnyansagar (1980) demonstrated that growth depression might be due to inhibition of auxin synthesis. Bansal *et al.* (1967) ascribed reduction in height to the shortening of internodes. Therefore, reduction in the growth of plant might have occurred due to inhibitory

effect of mutagens on growth regulating substances responsible for cell division and cell elongation. Ansari and Siddiqui (1995) reported that injury caused to the meristematic cells may be responsible for reduction in growth. Kumar and Tripathi (2008) are of opinion that the reduction in plant height may be attributed to chromosomal abnormalities after the treatment of mutagenic chemical.

The facts remain that the chromosomes, carrying various genes responsible for the life process and expression, are one of the most sensitive elements and the damage to any part of these vital and tiny elements are bound to go a long way to bring about various physiological and metabolic disorders, which in turn bring about several morphological and growth abnormalities in the plant or plant organs (Tabassum, 2002). This may be supported by the occurrence of various chromosomal abnormalities induced in *Capsicum annuum* L. by MMS and DES in present investigation. Therefore, it may be concluded that the chromosome and gene mutations are the causes of reduced germination and growth of seedlings and mature plants along with the physiological reasons as discussed above.

5.1.5 Days to flowering and Days to Maturity:

Mean days to flowering decreased in lower concentration and increased significantly in higher concentration of both the mutagens in both the varieties. Reduction in flowering/maturity time after mutagenic treatments has also been reported by different workers (Singh *et al*, 2000b; Waghmare and Mehra, 2000; Jabeen and Mirza, 2002, 2004; Patil 2009; Kozgar and Khan, 2009; Dhakshanamoorthy *et al*, 2010; Sonone *et al*, 2010; Aslam *et al*, 2012). Oka *et al.* (1958) reported that the average number of days to flowering was not altered much in some of the treatments indicating that mutations in major and minor genes had been in both the directions i.e., earliness as well as for lateness. Satyanarayana *et al.* (1993) reported increase in flowering time in M_3 than in M_2 generation and attributed this to the elimination of inferior lethal genes and also to the fixation of favourable genes for this trait in the populations. In the present investigation early flowering might be due to physiological changes in production of flowering hormones caused by the mutagens. Delayed flowering/maturity in higher concentrations as observed in the present study might be due to insufficient production of hormones required for

flowering. This is in favour of earlier work by Tripathi et al., (1975) in pigeonpea, Kothekar and Kothekar (1992) in moth bean, Panchbhaye (1997) in sunflower, Jayakumar and Selvaraj (2003) in sunflower, Manjaya and Nandanvar (2007) and Tambe (2009) in soybean.

Variation in flowering and maturity period in induced early mutants is generally considered to have parallel relation. However, early flowering mutants that are not early maturing (Tedin, 1954) or early maturity mutants with normal flowering date have also been reported in different crops (Porsche, 1963).

5.1.6 Yield:

Yield is a very important parameter in mutation breeding, because ultimately the plant breeder wants to improve the yield. In the present investigation yield decreased with increasing concentrations of MMS and DES in both the varieties, but an increase in yield per plant was observed in some lower concentrations of both the mutagens in var. Pusa jwala and var. G4. The yield parameters, such as number of fruits per plant and fruit size (fruit length and fruit diameter) also increased in the lower concentration of MMS and DES in both the varieties.

Decreasing trend in yield parameters has also been reported as result of the treatment with different mutagens by various workers such as Reddy and Rao (1982) and Lakshmi *et al.* (1988) in *Capsicum annuum*, Temple (1990) in *Lycopersicon esculentum*, Zeerak (1990) in *Solanum melongena* L., Maheshwari and Chand (1991) in *Hyoscyamus muticus*, Jain and Agarwal (1993) in *Trigonella foenum-graecum*, Kumar et al. (1993) and Khan et al. (2005a, b, 2006a, b) in Vicia *faba*, Khalil (2001) in *Carum carvi*, Tabassum (2002) in *Ammi majus*, Banu et al. (2005) in *Vigna unguiculata*, Pavadai and Dhanavel (2004) and Karthika and Lakshmi (2006) in *Glycine max*, Khursheed et al. (2009) in *Helianthus annuus* etc., while the increase in yield in lower concentrations of mutagen have earlier been recorded in a number of crops such as triticale (Viswanathan et al., 1994), *Vicia faba* (Vandana & Dubey, 1988 & Khan et al., 2005a, b, 2006a, b), *Lens culinaris* (Verma et al., 1999), *Triticum aestivum* (Kalia et al., 2000), *Trigonella foenumgraecum* (Jabee et al., 2007), *Helianthus annuus* (Khursheed et al., 2009), *Arachis hypogaea* L. (Sonone et al., 2010), *Vigna radiata* and *Vigna mungo* (Kozgar et al., 2011) *Cichorium intybus* L.

(Aslam *et al.*, 2012) etc. The reason for the increased yield in lower concentrations may be attributed to the enhancing effect (Jahagirdar, 1975; Kothekar, 1983) and growth regulatory effect of mutagen (Audus, 1961). The decrease in yield occurred due to induced disturbances in meiosis which affected the frequency of normal microspores upto greater extent and the megaspores to a lesser extent and hence the fruit set was directly affected. Singh and Chowdhury (1972) are also of the opinion that various chromosomal abnormalities are related with lower pollen fertility and ultimately the lower seed set. As the plant sterility was directly proportional to the administered doses and concentrations of mutagens, it can further be presumed that the mutagenic treatments affected both the male and female gametophytes more or less similarly. However, pollen sterility appeared to be more responsible, because the yield decreased under the condition of high pollen sterility (Lakshmi et al., 1988). This effect may also be interpreted as due to detrimental mutations, which are supposed to occur more frequently (Khamankar, 1974). The reason for the reduction in yield in higher concentrations of MMS and DES in the present investigation may be due to highly genotoxic nature of the mutagens which might have resulted in chromosomal damage physiological disturbances, failure or restricted pairing, delay in DNA synthesis and/or disturbed spindle formation and high pollen sterility.

Though there was general trend of decrease in yield in *Capsicum annuum* L, but some positive mutants showing higher yield were isolated in M_2 and M_3 generations. The increase in mean values for quantitative traits could be due to the occurrence of polygenic mutations with cumulative effects. Similar observation was also recorded by Singh *et al.*, (2000b).

5.2 Mutagenic Effectiveness and Efficiency:

The usefulness of a mutagens depends both on its effectiveness and efficiency, efficient mutagenesis being production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness is a measure of frequency of mutations induced by unit dose of mutagen, whereas, mutagenic efficiency is indicative of proportion of mutations as against undesirable biological effects such as gross chromosomal aberrations, lethality and sterility (Konzak *et al.*, 1965; Nilan, 1967).

Mutagenic effectiveness and efficiency has been worked out in large number of plants by many workers e.g., lentil (Sharma and Sharma, 1981a), *Catharanthus roseus* (Bhattacharjee *et al.*, 1998), blackgram (Khan, 1999), *Vigna mungo* L. (Deepalakshmi and Kumar, 2003), *Vigna unguiculata* L. (Dhanavel et al 2008), *Cyamopsis tetragonoloba* L. (Dube et al 2011) and *Lathyrus sativus* L. (Tripathy et al, 2012). Prasad (1972) and Nerker (1977) studied the effectiveness and efficiency of various mutagens and concluded that alkylating agents were more effective and efficient in inducing mutations. Furthermore, the lower doses of mutagens were more efficient and effective as compared to the higher doses. It was also supported by many worker such as Mitra & Bhowmik (1999) in *Nigella sativa* L., Waghmare & Mehra, (2001) *Lathyrus sativus* L., Singh & Singh (2001) in *Vigna radiata* L., Sharma et al. (2005) in *Vigna mungo* (L.) Hepper, Khan and Tayagi (2010) in *Glycine max* (L.) Merrill., Dube et al (2011) (*Cyamopsis tetragonoloba* L. and Satpute et al (2012) in *Glycine max* (L.) Merrill.

In the present study, the mutagenic effectiveness decreased with increasing concentrations of MMS and DES in both the varieties. The order of mutagenic effectiveness as determined on the basis of mutated plant progenies in M_2 generation was MMS>DES in varieties Pusa jwala and G4. The decline in the mutagenic effectiveness at higher concentration may be due to elimination of highly affected seedlings or plants at an early stage. Therefore it was found to be inversely proportional to the increasing concentrations of mutagens. Moreover, MMS was more effective than DES. The orders of mutagenic efficiency with regard to inhibition in germination (Mp/I) were MMS>DMS and MMS>DES and the orders of efficiency with regard to pollen sterility (Mp/S) were also MMS>DES in varieties Pusa jwala and G4 respectively. MMS was generally more efficient. Similar results have been reported in various crops by several workers (Waghmare & Mehra, 2001; Sharma et al., 2005; Shah et al., 2006). Solanki and Sharma (1994) considered that the higher efficiency of a mutagen indicates relatively less biological damage (i.e. seedling injury, sterility etc.) in relation to induced mutation.

5.3 Cytological analysis:

Cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the dependable indices to estimate the potency of a mutagen. Therefore, investigations on disturbances in meiotic behaviour indicating mutational genetic load form an integral part of most of mutation studies. It also provides a considerable clue to assess the sensitivity of plants for different mutagens and to ascertain the most effective mutagen for a given crop to realize maximum results.

In the present investigation a vast array of meiotic aberrations were recorded in the plants raised from the seeds treated with different concentrations of MMS and DES. The different types of chromosomal aberrations viz. univalents, multivalents, stickiness, precocious separation, chromatin bridges, laggards, and unequal separation, disturbed polarity, micronuclei and cytomixis were observed in the present investigation. Similar results were also reported by many workers in different plants after treatments with physical and chemical mutagens, viz., Anis and Wani (1997) in *Trigonella foenum-graecum*; Dhamyanthi and Reddi (2000) in *Capsicum annuum*; Singh (2003) in *Vigna radiata*; Rao and Laxmi (1980) and Katiyar (1978) in *Capsicum annuum* L., Singh and Chaudhary (2005) *in* Chilli; Bhat et al. (2005 a) in *Vicia faba*, Kumar and Singh (2003) in *Hordium* vulgare; Kumar and Dubey (1998) in *Lathyrus sativus*; Khan and Goyal (2009) in *Vigna mungo;* Alka *et al (2012)in Linum Usitatissimum L* and Aslam *et al (2012)* in *Cichorium intybus L.* Most of these worker have obtained a dose dependent increase in the meiotic aberrations.

The frequency of univalents ranged from 2-16 per PMC and these were later found as laggards at anaphase and telophase stages. The univalents were also reported by Saha and Datta (2002) in *Nigella sativa*, Kumar et al. (2003) in *Lens culinaris*, Sengupta and Datta (2004) in *Sesamum indicum* L., Bhat et al. (2006) in *Vicia faba* L., Khan and Goyal (2009) in *Vigna mungo* L., Aslam et al (2012) in *Cichorium intybus* L. It seems more likely that mutagens induced univalent formation through cryptic structural changes in some of the chromosomes which restricted pairing between homologous chromosomes and in this way reduced chiasma frequency.

According to Zeerak (1992) the mutagen-induced structural changes in chromosomes and gene mutations might be responsible for the failure of pairing

among homologous chromosomes resulting the occurrence of univalents. Previously it has been reported that the presence of univalents at metaphase-I might be due to asynapsis (lack of chromosome pairing during the late prophase-I), so that the homologous chromosomes failed to pair (Kaltsikes, 1973, Gustafsson, 1983) or desynapsis (inability to retain chiasmata in synapsed homologous chromosomes) resulting in premature separation of bivalents, so that the separated chromosomes will not be able to orient themselves at equatorial plate (Tsuchiya, 1970). Koduru & Rao (1981) are also of the opinion that the univalents occur due to asynaptic or desynaptic genes in prophase-I. Gottschalk and Kleine (1976) explained that the chromosome pairing is under the control of 2 groups of genes viz. *As* and *Ds* which when present in recessive state, may cause chromosome pairing to fail. The emergence of univalents could also be due to precocious chiasma terminalization (Gottschalk & Kaul, 1980b; Sidhu, 2008). Thus, reduced chiasma frequency as a result of increased heterology may be one of the reasons of increased number of univalents with increasing concentration of mutagens (Gottschalk & Kaul, 1980a; Jabee & Ansari, 2005). The absence or highly reduced number of univalents in M3 generation was due to the fact that desynapsis or asynapsis did not occur due to the ceasing effect of mutagens and the normal pairing of bivalents. It may also be due to repair mechanism in the case of damaged DNA. Some of these univalents were later on found to be laggards at anaphase and telophase stages. Thus, gamma rays, MMS and DES might have induced genic disturbances due to mutagenic activity and hence the disturbances in homology and pairing of homologous chromosomes.

Multivalent formation as observed in the present investigation has also been reported by many workers in several crops such as Zeerak (1992) in *Lycopersicon esculentum* and Siddiqui and Ansari (2005) in *Solanum melongena* L.; Kumar and Rai (2007a) in *Zea mays* L.; Kumar and Tripathi (2004) and Kumar and Gupta (2009) in *Capsicum annuum*, etc.

The multivalent formation was due to the breakage in chromosomes caused by these mutagens and their reunion through reciprocal translocations. Chghtai and Hasan (1979) recorded the multivalents with increasing dosage of EMS, MES and MMS in Lens esculenta and suggested that translocation might have been produced due to terminal affinities of broken chromosomes. Zeerak (1992), Vandana et al.

(1996), Kumar and Sinha (1991), Anis and Wani (1997) and Kumar and Gupta (2009) attributed the multivalent formation to irregular pairing and breakage followed by translocation and inversion. According to Lea (1955) and Srivastava (1979) the broken ends of the chromosomes when fused at random may bring about unequal changes making up the multivalents.

Stickiness could be due to depolymerisation of nucleic acid caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alteration in their pattern of organization (Evans, 1962). Jabee and Ansari (2005) suggested that chromosomal breakage may cause stickiness among the chromosomes. It may also be due to genetic and environmental factors (Rao et al., 1990; Nirmala & Rao, 1996; Souza & Pagliarini, 1996; Baptista-Giacomelli et al., 2000a). Stickiness could arise due to depolymerization of nucleic acid caused by mutagenic treatment (Tarar & Dnyansagar, 1980; Kumar et al., 2003; Kumar & Tripathi, 2003; Jabee et al., 2008) or due to dissociation of nucleoproteins and alteration in their pattern of organization (Katiyar, 1978; Myers et al., 1992; Kumar et al., 2003 & Kumar & Rai, 2007c). In the present case, MMS and DES seems to be responsible for induced stickiness, perhaps the target proteins in this case are those responsible for chromosome condensation during active divisional stages. Their defective functioning, which may be due to gene mutation or direct action of the mutagen on the proteins, caused a disturbance in the chromosomes during the course of their condensation from prophase-I to metaphase-I.

Precocious movement of chromosomes was frequently found in present investigation at metaphase-I and -II stages. Similar was reported by the work of Das and Roy (1989) in *Solanum sisymbrifolium*, Pagliarini (1990) in *Aptenia cordifolia*, Pagliarini and Pereira (1992) in *Pilocarpus penneatifolius Lem*, Anis and Wani (1997) and Siddiqui et al. (2007) in *Trigonella foenum-graecum*, Kumar and Tripathi (2004) in *Capsicum annuum*, Kumar and Rai (2007c) in *Glycin max*, Malik and Shrivastava (2007) in Carthamus, Bhat et al. (2007) and Khan et al. (1998a, 2007b, c) in *Vicia faba*, Defani-Scoarize et al. (1995a, b) and Kumar and Rai (2007a) in *Zea mays*, Khan et al. (2009a) in *Cichorium intybus* etc.

The precocious movement of chromosomes might have been caused by the early terminalization or stickiness of chromosomes and/or movement of chromosomes ahead of the rest during anaphase (Premjit & Grover, 1985). It may be due to the disturbed homology for chromosome pairing or disturbed spindle mechanism (Agarwal & Ansari, 2001), either because of the abnormal spindle activity (Amer & Ali, 1974; Umar & Singh, 2003; Kumar & Gupta, 2009) or due to the reunion of chromatids during meiotic prophase (Rees & Thompson, 1955; Lewis & John, 1966; Newmann, 1966).

The univalents separating precociously seemed to be as a result of desynapsis (Bose & Saha, 1970; Kaul & Nirmala, 1993, Kumar & Rai, 2007a) or asynapsis (Roy et al., 1971). Moreover, structural differences of homologous pairs followed with disturbed spindle mechanism might have resulted in haphazard movement of chromosomes, some of them being precocious.

Stray chromosomes were observed at metaphase-I which may be caused by spindle dysfunction and clumping of chromosomes. Similar results were also reported in *Vicia faba* L. (Bhat et al, 2007; Gulfishan et al 2010) and in *Hordeum vulgare* L. (Jafri et al., 2012).

Occurrence 0f laggards may be explained on the basis of abnormal spindle formation and chromosomal breakage. The laggards observed in the present study might be due to delayed terminalization, stickiness of chromosome ends or because of failure of chromosomal movement (Permjit and Grover, 1985; Jayabalan and Rao, 1987b; Soheir *et al.*, 1989).

Tarar and Dnyansagar (1980) and Das and Roy (1989) are of the opinion that due to the effects of mutagens the spindle fibres failed to carry the respective chromosome to the polar regions and resultantly the lagging chromosomes appeared at anaphase-I. According to Pagliarini (1990) laggards may be the result of late chiasma terminalization. Kumar and Rai (2007a, 2009) also support the opinion that laggards might have appeared due to improper spindle functioning. Kumar and Gupta (2009) reported that fragments which appeared on the breakage of bridges, as a result of spindle fiber functioning to pull the chromosomes towards the poles, formed laggards.

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Bridges with or without fragments at anaphase stages were observed in the present investigation. The bridges were also observed by Reddy and Annadurai (1992) in *Lens culinaris* by different mutagens, George and Abd El-Haleem (2001) in *Vicia faba* by uccmaluscide (molluscicide), Khan et al. (1998a, 2007b) in *Vicia faba* by caffeine, DES and 8-HQ, Kumar et al. (2003) in *Lens culinaris* by combined treatment of gamma rays and EMS, Jabee and Ansari (2005) in *Cicer arietinum* by hydrazine sulphate (HS), Kumar and Rai (2007a) in *Zea mays* By EMS, Siddiqui et al. (2007) in *Trigonella foenum-graecum* by Sodium azide (NaN3), Khan et al. (2009b) in *Cichorium intybus* by 2, 4-D, Singh and Chaudhary (2005) and Kumar and Gupta (2009) in *Capsicum annuum* L.

In the present study occurrence of bridge with fragment may be due to paracentric inversion. The present findings are in agreement with the earlier results of Jayabalan and Rao (1987) in tomato and Mitra and Bhowmik (1996) in *Nigella sativa*. Saylor and Smith (1966) suggested that the formation of chromatin bridges might be due to the failure of chiasmata in a bivalent to terminalise and the chromosomes get stretched between the poles. Bhattacherjee (1953) attributed bridge formation to interlocking of bivalent chromosomes and Sinha and Godward (1972) to paracentric inversions. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosome which is pulled equally to both the poles forming a bridge (Anis *et al.*, 1998). Bridges can also be attributed to the general stickiness of chromosomes at metaphase which further led to their inability to separate or to the breakage and reunion of chromosomes (Ahmad, 1993; Anis & Wani, 1997; Kumar & Gupta, 2009).

Unequal separation of chromosomes towards poles at anaphase due to non-disjunction of homologous chromosomes at metaphase as observed during the present study was due to the stickiness of chromosomes and could result in unequal distribution of chromosomes in the daughter nuclei (Anis and Wani, 1997). Mitra and Bhowmik (1996) reported that unequal separation of chromosomes was caused by spindle irregularities. Random movement of univalents to any one of the poles leads to unequal separation of chromosomes (Kumar and Singh, 2003). Kumar and Rai (2007c) reported that unequal separation of chromosomes in meiosis-I and -II might be the result of the non-oriented bivalent formation due to spindle dysfunction or due

to the formation of univalents at diakinesis or metaphase. It might have also occurred due to early or delayed separation of bivalents. Micronuclei generally arise from fragments and lagging chromosomes which fail to reach the poles and get included in the daughter nuclei (Kumar and Duby, 1998b). Laxmi *el al.*, (1975) suggested that irregular distribution of acentric fragments or laggards results in the formation of micronuclei at telophase resulting in variation in number and size of pollen grains obtained from the pollen mother cell. Micronuclei at dyad or tetrad stage of PMCs in mutagen treated population might have resulted due to non-orientation of chromosomes and laggards since they were of frequent occurrence. Micronuclei lead to the loss of genetic material. Their presence, therefore, suggests that the resultant product of meiotic division is deficient in one or the other chromosome. This usually leads to the formation of sterile pollen grains.

Disturbed polarity at telophase stages was recorded in certain percentage of the PMCs which could be due to spindle disturbance. Disturbed polarity was also reported by Sharma et *al.* (2004) in chickpea and Bhat *et al.* (2006) in *Vicia faba* L.

In the present investigation, very low frequency of pollen mother cells showed cytomixis at various stages of meiosis was noticed. Similar observation was reported by several workers during microsporogenesis (Soodan and Wafai, 1987; Bahal and Tyagi, 1988; Kaul 1990; Kumar and Sharma, 2002; Bhat et al., 2006; Kumar and Rai, 2007).

Among the factors proposed to cause the cytomixis are i) influence of genes (Kaul and Nirmala, 1993), ii) abnormal formation of the cell wall during premeiotic divisions (Kamra, 1960), iii) action of chemical agents such as colchicines, ethyl methane sulphonate and methyl methane sulphonate (Sinha, 1988; Bhat, *et al,* 2006) and rotenone (Amer and Mikhael, 1986), iv) changes in the biochemical process that involve microsporogenesis modifying the micro-environment of affected anthers (Kaul, 1990), v) effect of gamma radiation resulting in an unbalanced and sterile genetic system (Amma *et al.* 1990), vi) due to the presence of a male-sterile mutant gene and its frequency altered by environmental factors (Nirmala and Kaul, 1994) and environmental stress and pollution (Haroun *et al.*, 2004). In the present investigation the occurrence of cytomixis may be due to action of mutagens used (Sinha, 1988)

Cytomixis is considered to be of less evolutionary importance but it may lead to the production of aneuploid plants with certain morphological characteristics (Sheidai *et al.*, 1993) or produce unreduced gametes as reported in *Aegilops* (Sheidai *et al.*, 2002). Unreduced gamete formation is of evolutionary importance leading to the production of plants with higher ploidy levels. Cytomixis by producing aneuploid pollen grains may also be responsible for reduction in pollen fertility.

In the present investigation, a dose dependant decrease in the chiasmata frequency was observed in various treatments. The average frequency of chiasmata per cell decreased with an increase in univalents and rod bivalents in each treatment. The reduction in chiasma frequency can be attributed to an increase in rod bivalents and univalents (Anis *et al.* 2000). Similar observations were reported by Bennett and Rees (1970) in rye and Sadanandam and Subash (1984) in *Capsicum annuum*.

Chiasma formation characterizes the pairing of homologous chromosomes at meiosis and controls the degree of recombination. Chiasma counting is the most straightforward method of scoring the total no. of crossing-over events in the genome (Baptista-Giacomelli et al., 2000b). The decrease in the chiasma frequency denotes the induced heterology due to induced damage or changed loci of genes or intra/inter-genic disturbances following the mutagenic treatments. In the present study the decrease in chiasma frequency was relatively less in M2 and M3 generations in comparison to M_1 generation, indicating that some sort of recovery mechanisms must have been operating in the intervening period.

According to Lawrence (1961) the decrease in chiasma frequency following mutagenic treatments might possibly occur at two stages: (i) during DNA synthesis and (ii) during sensitive period at/or slightly before the stage of chiasmata formation. In the former case the decrease in frequency of chiasmata may be due to disturbances in chromosome coiling, restricted pairing at pachytene and the delay in DNA synthesis, while in the latter, it may be affecting the process leading to chiasmata formation. Sadanandan and Subhash (1985) attributed it to the nature and potency of mutagens and also the underlying factors, such as complex structural changes or the changes in the nature of gene responsible for chiasmata formation. The alteration of chiasmata in the treated plants might also be due to the failure of complete pairing

(Anis and Sharma, 1997) or rapid terminalization of chiasmata in the bivalents (Tabassum, 2002). The decrease in chiasma frequency may also be attributed to the changes at chromosomal/DNA level, such as deletion, inversion, duplication and translocation (Siddiqui and Ansari, 2005).

In the present investigation, the isolated mutants were analyzed for capsaicin content. Increased capsaicin content mutant, after mutagenic treatment, was isolated by Bansal (1969) in var. NP46 while Abdul Salam and Thoppil (2010) isolated 6 mutants in *Capsicum annuum* L. treated with Ethyl methane sulphonate and Sodium azide with varying capsaicin content. Small sized fruits contain high concentration of capsaicin while long fruits contain low concentration of capsaicin (Abdul Salam and Thoppil, 2010). Fruits with thin pericarp contain higher capsaicin content as compared with varieties having thick pericarp (Hosmani, 1993). The capsaicin content is also dependent upon placental content of chilli fruits, more the placental tissue higher the capsaicin content (Ramanujan and Thirumalachar, 1966). The change in capsaicin content in chilli is also affected by change in climate (Tiwari et al., 2005). In the present study the degree of pungency varies, probably due to action of mutagenic treatments on genes (*Pun 1*) which is a modifying factor for pungency (Stewart et al., 2005).

On the basis of the present investigation and discussion it has been concluded that the cytomorphological variations observed in the present study are due to the above mentioned genic disturbances induced by the action of chemical mutagens, along with their interactions with environment. The physiological, biochemical and metabolic changes might have indirectly affected the treated plants due to the disturbances at chromosomal and genic level. But selfing each variant in M_1 followed by selection and selfing in M_2 and M_3 generations eliminates upto maximum extent, the possibility and overlapping role of other factors and concentrates to stable genetic changes in the mutants obtained.

It has also been revealed that lower and moderate treatments of mutagens used in the present investigation proved to be efficient in increasing the genetic variability for yield-oriented selection in *Capsicum annuum* L. The isolated mutants possess desirable plant architecture associated with high yield and variable capsaicin content

than their respective controls. They can be evaluated in future generations and after multilocational trails released as new varieties. The meiotic aberrations indicate the mutational load. The frequency of meiotic aberrations decreased from M_1 to M_2 and M_2 to M_3 and further decreased in isolated strains which represent the occurrence of stability in mutated genotype from M_1 to M_3 generation and in the isolated mutants of both the varieties of *Capsicum annuum* L. Thus the induced cytomorphological variations in the present investigation have provided greater chances of selection for different desirable characters and may play an important role in increasing the diversity in *Capsicum annuum* L.

Chapter-6

Summary and Conclusion

SUMMARY AND CONCLUSION

Crop plants form the major components of human diets, providing the required calories and nutrients to sustain life. With recent soaring of food prices, which is one of the immediate causes of the current food security crisis, the need to efficiently increase food availability through the production of high yielding crop varieties under the contrary effect of climate change and variability, plays a key role in ensuring food security.

An essential aspect of crop improvement is the utilization of the available genetic variation to produce new crop varieties. Induced mutations are a proven tool in creating a wealth of desirable genetic variability in plants, and its success in crop improvement abound. It is an effective tool in hands of plant breeders specially in crops having narrow genetic base. The widespread use of induced mutants in plant breeding programme throughout the world has led to the official release of more than 3000 mutant plant varieties. Many mutants have been identified as donor of desirable traits in breeding programme. It is noteworthy that a large number of mutant varieties had been developed and widely cultivated in developing countries, hence greatly improving food security in those countries.

Capsicum annuum L. is usually grown as a herbaceous annual in temperate areas. However, ecologically it is a perennial shrub in tropical areas, and it can be grown as a perennial in climate-controlled greenhouses. It is an indispensable vegetable cum spice used as basic ingredient in a great variety of cuisines all over the world. It is also used as flavourant, colourant and adds tang and taste to the otherwise insipid food. *Capsicum* species are employed whole or ground and alone or in combination with other flavouring agents, primarily in the pickles, stewed or barbequed. The nutritive value of *Capsicum* is high and it is an excellent source of vitamins C (ascorbic acid), A, B-complex and E along with minerals like molybdenum, manganese, folate, potassium and thiamine. Chilli contains seven times more vitamin C than orange. Beta-carotenoids, and vitamins C and A in chillies are powerful antioxidants that destroy free radicals. The therapeutic properties and pungency exhibited in *Capsicum* is attributed to a group of alkaloids known as capsaicinoids. The hotness of the chilli is mainly because of capsaicin ($C_{18}H_{27}NO_3$),

which is a condensation product of 3-hydroxy, 4-methoxy benzyl amino and decylenic acid. As a medicine it is used as a counter irritant in lumbago, neuralgia, rheumatic disorders and non-allergic rhinitis. It has a tonic and carminative action. In combination with cinchona it is employed in intermittent and lethargic afflictions and also in atonic gout, dyspepsia accompanied by flatulence, tympanitis and paralysis. Its most valuable application appears in cynanche maligna and scarlatina maligna used either as a gargle or administered internally. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps and toothache.

The present investigation was carried out to study the mutagenic effects of methyl methane sulphonate and diethyl sulphate on cytomorphological parameters of two varieties of *Capsicum annuum* L. viz., var. Pusa jwala and var. G4. The parameters taken into consideration were Seed germination, plant survival, pollen fertility and meiotic behaviour of chromosomes in M_1, M_2 and M_3 generation. Seven quantitative characters viz., plant height, days to flowering, days to maturity, fruits per plant, fruit size and yield per plant were also studied in M_1, M_2 and M_3 generation. The findings are as follows.

- Seed germination and pollen fertility showed a dose dependent decrease with MMS and DES in varieties Pusa jwala and G4. Similar trend was followed in these doses in M_2 and M_3 generations also but considerable recovery occurred in these parameters. The maximum reduction in seed germination and pollen fertility was found in the highest conc. of MMS.

- Plant survival decreased with increasing concentrations of mutagens in both varieties. The maximum plant survival was observed in DES than MMS in both the varieties. The plant survival was higher in M_2 and M_3 generations than M_1 generation.

- The average height of seedlings and mature plants in M_1 generation decreased with increasing concentrations of MMS and DES in both varieties. The maximum reduction in seedling as well as mature plant height was obtained in MMS followed by DES in varieties Pusa jwala and G4. The M_2 and M_3 generation exhibited recovery in the average height of mature plants in general treated populations. Moreover, some mutants obtained as a result of

segregation in M_2 and M_3, following the expression of new genes, were dwarf and some taller than control plants.

- Frequency of variations increased with increasing concentrations of both the mutagens in both varieties. The mutants in M_2 were selected on the basis of selfing the variants of M_1. The frequency of mutations was generally lower in M_2 and M_3 generations, than those obtained in M_1 generation.

- Various micro mutational characters such as average number of fruits, fruit length and diameter, and total yield per plant were studied in M_1, M_2 and M_3 generations. In M_2 generation the mutation frequency was observed maximum in MMS followed DES. The trend in M_3 generation is similar to M_2. Generally the yield was higher in M_2 and M_3 generations than M_1 due to the ceasing toxic effect of mutagens.

- The mutagenic effectiveness calculated in M_2 generation was inversely proportional to the increasing concentration of both the mutagen. The effectiveness was highest in MMS followed by DES. Efficiency was worked out on the basis of seedling injury (Mp/I) and pollen sterility (Mp/S) and showed a decreasing trend with increasing concentrations of mutagens. The order of efficiency was MMS> DES.

- The effect of mutagenic treatments on meiosis was studied in detail. The chromosomal aberrations induced by MMS and DES were univalents, multivalent, stickiness, precocious separation, stray bivalents at metaphase I/II. The induced chromosomal aberration at anaphase I/II were laggards, bridge with or without fragments, unequal separation and non synchronization, while at telophase I/II the induced chromosomal aberrations were cytomixis, micronuclei, disturbed polarity and bridges. Chromosomal aberrations were same in M_1, M_2 and M_3 generations, but their frequencies were lesser in M_2 than M_1 and in M_3 lesser than M_2.

- The chiasma frequency (per cell and per bivalent) generally decreased with the increasing concentrations of mutagens at metaphase-I. The maximum adverse

effect on chiasma frequency was caused by MMS than DES treatments. The similar pattern was followed in M_2 and M_3 generations also, but with slight recovery.

- The results of these chromosomal variations was obtained in the form of mutations The positive mutants have been isolated in M_3 showing various morphological characters almost similar to those in M_2 such as tall, dwarf, high or low yielding etc.
- On the basis of yield performance, mutants isolated in M_2, were evaluated in order to find out the selection response in M_3 generation. A considerable increase in yield per plant was recorded in almost all the mutant lines except the early maturing mutants where the yield was normal as control.
- The capsaicin content of all high yielding mutants in M_3 generation was estimated by using HPLC technique. The capsaicin content in some of the mutants was higher than control while in some others it was lower than control.

It has been concluded that morphological and cytological variation observed in the present investigation due to chromosomal/genic mutations leading to physiological disturbances in metabolic activities or growth regulators or gene expression and finally the production of mutants. It can be presumed that wherever enzymes are involved there must be the involvement of genes, as the genes are expressed in the forms of proteins and enzymes. If there is any alternation at genic or base level the mutation is bound to occur.

Moreover, the isolated mutants with respect to morphological traits, yield and yield components will be useful in the improvement of *Capsicum annuum* L., and should be further evaluated in the subsequent generations for the isolation of stable high yielding progenies. These progenies with improved yield performance may be multiplied for further use. Thus, the induced cytomorphological variabilities in the present investigation provided greater chances of selection for different desirable character in *Capsicum annuum* L.

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